資料３

# 感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

## １　基本的な方針

運営委員会に報告する資料においては、

(1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。

(2) ８月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとすること。

## ２　具体的な方法

(1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。

① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第２）及び該当文献を添付した「資料概要Ａ」を事務局が作成し、送付する。

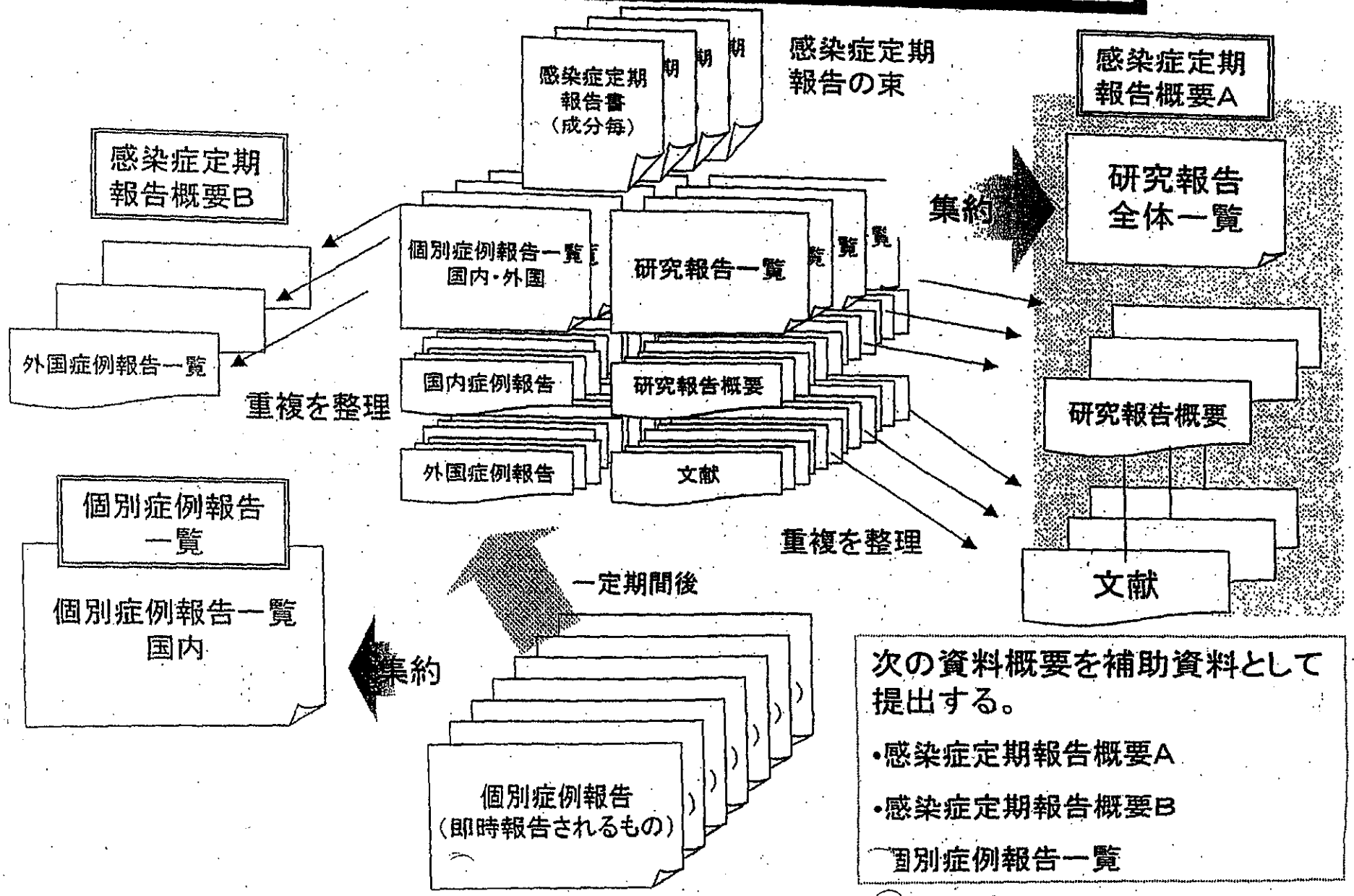
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第４）をまとめた「資料概要Ｂ」を事務局が作成し、送付する。

③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例に係る「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。

(2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。

(3) 資料概要Ａ及びＢについては、平成１６年９月の運営委員会から試験的に作成し、以後「感染症定期報告について（目次）」資料は廃止することとする。

# 感染症定期報告・感染症個別症例報告の取り扱い




次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

資料３

# 感染症定期報告概要

（平成１９年７月２５日）

平成１９年３月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

# Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成１９年３月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

２　一覧表においては、前回の運営委員会において報告したもの以降の研究報告について、一覧表の後に当該感染症の主要研究報告の内容を添付した。

# 感染症定期報告の報告状況(2007/3/1～2007/5/31)

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
|---|---|---|---|---|---|---|
| 60248 | 2007/03/30 | 61061 | A型肝炎 | J Med Virol 2006; 78: 1398-1405 | A型肝炎ウイルス(HAV)感染患者の血液および糞便中へのウイルス排泄期間および排泄量と、アラニンアミノトランスフェラーゼ(ALT)、疾患重症度、HAV遺伝子型との関連を調べた。27例の急性HAV患者でHAVは発症後81日間(中央値)便中に排泄され、半数で36日目でも多量なウイルスの排泄が続いた。ウイルス血症は検出されたが、定量できなかった(中央値42日間)。疾患発症後10日間は、ALT値が高いほど血中ウイルス量が高かった。遺伝子型1aと1bの患者で、HAV排泄および黄疸の期間に有意差はなかった。 | 1 |
| 70057 | 2007/05/29 | 70215 | A型肝炎 | J Med Virol 2007; 79: 356-365 | 1997-2005年に、デンマーク、ドイツ、オランダ、ノルウェー、スペイン、スウェーデンおよび英国で、男性同性愛者にA型肝炎が大流行した。このA型肝炎アウトブレイクに関連する株の遺伝子学的関連性を調べたところ、これらの国の男性同性愛者から得られた株の大部分はMSM1と名づけられた遺伝子型IAに属する近縁のクラスターを形成していた。同期間に他のリスク群では異なったHAV株が流行していたことから、特異的な株がヨーロッパの男性同性愛者間では流行していたことを示す。 | 2 |
| 60248 | 2007/03/30 | 61061 | BSE | ABC Newsletter 2006年9月22日 16ページ | 欧州協議会は2005年の反芻動物(有蹄動物)における伝達性海綿状脳症(TSE)のモニタリングと検査に関する報告書を発表した。TSE検査を行った1千万頭以上のウシのうち、陽性となったのは561頭のみであった。2005年の調査結果は陽性例が引き続き減少していることを示している。 | |
| 70021 | 2007/04/25 | 70125 | BSE | Canadian Food Inspection Agency 2007年2月7日 | 2007年2月7日、CFIAはAlbertaの成牛はBSEであると確定した。カナダにおける9頭目のBSE牛である。死体は管理され、どの部位もヒト食料または動物の餌システムに入っていない。予備的情報ではこのウシは生後1年目に少量の感染物質に暴露したと考えられる。国際的ガイドラインに従った疫学的調査が開始された。 | 3 |
| 70021 | 2007/04/25 | 70125 | BSE | FDA News; P07-04, 2007年1月11日 | FDAはBSEセーフガードとして医薬品や医療機器で、特定のウシ原料を禁止することを提案した。禁止される原料は、30月齢以上のウシの脳、頭蓋骨、眼および脊髄、全てのウシの扁桃腺および小腸の一部、ヘタリウシの全ての部位、検査を合格していないウシの全ての部位などである。 | 4 |
| 70021 | 2007/04/25 | 70125 | BSE | PLoS Pathogens 2006; 2: 956-963 | 最近、大規模なスクリーニングによって、従来とは異なるPrPresがウシにおいて発見された。H型と呼ばれる高分子量のフランスのウシPrPres分離株を、ウシまたはヒツジのPrPを発現するトランスジェニックマウスに接種した。全てのマウスは神経学的症状を呈し、死亡し、これらの株が感染性プリオンの新規株であることが示された。この病原体は、BSE病原体およびヒツジスクレイピー病原体とは明らかに異なる特有の神経病理学的特徴を示した。 | 5 |
| 70057 | 2007/05/29 | 70215 | BSE | ProMED-mail20070302.0734 | ニュージーランド食品安全局はBSEを取り巻く最新の科学と実際の知識を踏まえて、ウシ及びウシ加工品の輸入規制を改訂する方針である。新しい規制は科学的証拠や最近の国際的な規制に合致したものとするため、輸出する国のBSEリスクステータスの分類に、国際的に認められた3カテゴリーシステムを導入する。ゼラチンは、原材料の起源およびBSEリスクのある国からの輸入を問わず、全てのゼラチンの売買が自由化される。 | 6 |
| 70017 | 2007/04/25 | 70121 | B型肝炎 | EMEA/CHMP/BWP/298390/2005 2006年9月21日 | EMEAによる、血漿プール中のB型肝炎ウイルス表面抗原(HBSAG)検出のためのイムノアッセイの確認に関するガイドライン。市販キットを使用する際の注意事項、血漿プール試験のためのSOPなどについて述べられている。 | 7 |

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|---|---|---|---|---|---|---|
| 60248 | 2007/03/30 | 61061 | B型肝炎 | Transfusion 2006; 46: 2028-2029 | 2004年10月、神奈川県赤十字血液センターは輸血後HBV感染疑い症例の報告を受けた。供血当時の検査では50プールNAT陰性だったにも関わらず、凍結検体がHBV個別NAT陽性となった供血者を特定した。この供血者の凍結血液40検体について個別NATを行ったところ、陰性と陽性があった。合計6例の輸血後HBV感染が特定された。この供血者におけるHBV DNAの量は50コピー/mL未満から200コピー/mLの間で増減していた。供血前に個別NATを行ったとしても、全てのHBVキャリアを排除できないことが示された。 | 8 |
| 60248 | 2007/03/30 | 61061 | B型肝炎 | Vox Sang 2006; 91: 237-243 | ドイツ赤十字血液センターの供血者10000名を、現行のPRISM® HBcおよび新規PRISM®Hbcore検査を用いたHBc抗体のスクリーニングを実施し、診断感度および特異性を調べた。両者とも約1.8%がHBc抗体陽性であることを示し、感度は同等であったが、特異性はPRISM® Hbcoreの方が有意に高かった。この検査が陽性であった188検体について、さらに7種類の抗HBc検査、2種類の抗HBs検査、1種類の抗Hbe検査、3種類のHBV NAT検査を行って、検査結果を比較した。 | |
| 60248 | 2007/03/30 | 61061 | B型肝炎<br>C型肝炎 | Transfusion 2006; 46: 1997-2003 | 健康歴の問診によって供血延期となった供血者497名を、4つの米国赤十字血液センターで募集し、血液感染症の血清マーカーについて血液検体を検査した。その結果、ウイルス肝炎リスクおよび静注薬物使用歴に関する標準的な供血者用問診にて供血停止となった供血者は、供血停止とならなかった供血者よりも肝炎マーカー陽性率が高い場合が多かった。その他のマーカーおよび質問について有意な知見は認めなかった。 | 9 |
| 70034 | 2007/04/27 | 70150 | B型肝炎<br>C型肝炎 | Transfusion 2006; 46: 1997-2003 | 健康歴の問診によって供血延期となった供血者497名を、4つの米国赤十字血液センターで募集し、血液感染症の血清マーカーについて血液検体を検査した。その結果、ウイルス肝炎リスクおよび静注薬物使用歴に関する標準的な供血者用問診にて供血停止となった供血者は、供血停止とならなかった供血者よりも肝炎マーカー陽性率が高い場合が多かった。その他のマーカーおよび質問について有意な知見は認めなかった。 | 10 |
| 60248 | 2007/03/30 | 61061 | C型肝炎 | JAMA 2006; 296: 2005-2011 | 2004年10月15日にメリーランドで放射性医薬品注射剤を用いて心筋灌流試験を行った患者16名に発生した急性HCV感染について調べた。患者はある薬局で調整された1つのバイアルの注射剤を投与されていた。その薬局では、注射剤を調製する12時間前に、HCVおよびHIVに罹患した患者の血液の放射線標識白血球測定を行っていた。この患者から得られたHCVのシークエンスは、当該16症例の配列とほぼ同一であった(相同性97.8%～98.5%)。生物由来製剤を取り扱う放射性医薬品薬局は、適切な無菌操作を行うべきである。 | 11 |
| 60234 | 2007/03/23 | 61006 | E型肝炎 | 肝臓 2006; 47: 384-391 | わが国のE型肝炎の実態を明らかにする目的で、全国から総数254例のE型肝炎ウイルス感染例を集め、これを解析した。その結果、以下の知見を得た。1)HEVは全国に浸透している。2)感染者の多くは中高年(平均年齢約50歳)で、男性に多い。3)我国に土着のHEVの遺伝型は3型と4型である。4)年齢と肝炎重症度に相関がある。5)遺伝型は4型が顕在化率も重症化率も高い。6)発症時期が無季節性である。7)感染経路は、動物由来食感染が約30%、輸入感染が8%、輸血感染が2%、不明が約60%であった。 | |
| 70048 | 2007/05/22 | 70198 | G型肝炎 | Epidemiol Mikrobiol Imunol 2006; 55: 136-139 | チェコ共和国における静注免疫グロブリン(IVIG)投与患者の血清中におけるHGV陽性率を調査し、HGV陽性に関係したリスクを検討した。IVIG投与患者86例の内20例(23%)が、HGV RNA陽性であった。その内3例には肝機能検査値の緩やかな上昇が認められ、また1例は慢性リンパ性白血病であったが、IVIG投与前に診断されていた。IVIG投与患者のHGV感染率は高いが、肝疾患又はリンパ増殖のいずれの兆候とも関連していないと結論付けられる。 | 12 |

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|---|---|---|---|---|---|---|
| 60237 | 2007/03/27 | 61024 | HHV-8感染 | N Engl J Med 2006; 355: 1331-1338 | 2000年12月から2001年10月に輸血を受けたウガンダのKampalaの患者1811例のうち、輸血前にヒトヘルペスウイルス8型(HHV-8)血清陰性であった患者991例について追跡調査を行った。そのうち43%(425例)にHHV-8血清陽性血が輸血された。991例中41例にHHV-8セロコンバージョンが起こったが、セロコンバージョンのリスクは陽性血を輸血された患者の方が陰性血を輸血された患者より有意に高かった。 | |
| 70017 | 2007/04/25 | 70121 | HIV | EMEA/CHMP/BWP/298388/05 2006年9月21日 | EMEAによる、血漿プール中の抗HIV抗体検出のためのイムノアッセイの確認に関するガイドライン。市販キットを使用する際の注意事項、血漿プール試験のためのSOPなどが述べられている。 | 13 |
| 70057 | 2007/05/29 | 70215 | HIV | Lancet 2007; 369: 621-623 | 2002年の国連レポートや米国国家情報会議は、中国には約100～200万人のHIV/AIDS患者がおり、感染爆発の危機が迫っているとしたが、2006年までの生存患者数は65万人と見積られた。感染規模の過大な予測から、中国では様々な問題が生じた。<br>HIV/AIDS対策に多大な予算を掛けたために、喫煙、結核など他の健康問題への対策が十分ではなかった。中国でのHIV/AIDS対策はハイリスク地域を中心に行うべきである。 | 14 |
| 70006 | 2007/04/23 | 70089 | HIV | Vox Sanguinis 2007; 92: 113-120 | 20例の血友病患者が、1990年初頭以降、韓国で製造された血液凝固第IX因子の投与を受けてから1～2年後にHIV-1に感染していると診断された。血漿ドナーと血友病患者で検出されたウイルス間の遺伝子関連性を調べた結果、両者とも、HIV-1サブタイプBの韓国subcladeに感染していた。韓国で売血ドナーの血液から製造された凝固因子により、少なくとも20例の血友病患者がHIV-1サブタイプBに感染したことが明らかとなった。 | 15 |
| 60248 | 2007/03/30 | 61061 | HIV | 中日新聞 Chunichi Web Press 2006年9月4日 | エイズウイルス(HIV)のうち、世界で感染が広がっている主流のHIV1型とは遺伝子タイプが異なる2型に日本人が初めて感染したことを、厚生労働省のエイズ研究班が確認したことが9月3日分かった。厚労省は、医療機関や保健所などが実施している検査で2型の感染を見逃さないよう、検査の徹底を求める通知を出した。<br>HIV2型の感染が確認されたのは、過去に西アフリカで輸血を受けた経験がある男性である。同省は「滞在していた地域では2型が流行しており、現地での輸血が感染原因とみられる」としている。 | |
| 60237 | 2007/03/27 | 61024 | HIV | 日刊薬業 第12105号 平成18年9月6日 | 日本人初のHIV-2感染者が確定された。男性は過去に西アフリカに渡航し、現地で輸血した経験があるため、これが感染経路と見られている。厚労省は、2型の検査も確実に行い、検査漏れがないよう、各都道府県に通知した。 | |
| 60234 | 2007/03/23 | 61006 | アルツハイマー型認知症 | Science 2006; 313: 1781-1784 | アルツハイマー病患者、またはβ-アミロイド前駆体タンパク質(APP)発現トランスジェニックマウスから得たアミロイド-β(Aβ)含有脳抽出物の希釈液を大脳内に注射すると、APPトランスジェニックマウスに、時間と濃度に依存した大脳内のβ-アミロイドーシスとそれに伴う病変を誘発した。脳抽出物のシーディング活性は、Aβ免疫除去、タンパク変性、またはAβを宿主に免疫することによって、低下または消失した。外因性に誘発させたアミロイドーシスの表現型は、宿主と誘導物質の両者に依存した。 | 16 |

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| 70023 | 2007/04/26 | 70137 | インフルエンザ | Science 2007; 315: 655-659 | 1918インフルエンザウイルスのヘマグルチニン受容体結合部位のごくわずかな変化により、ウイルスの伝播性が変化することが示された。2つのアミノ酸変異によって、ヒトのα-2,6シアル酸からトリのα-2,3シアル酸へと転換すると、フェレット間で呼吸器飛沫による感染を起こさないウイルスとなった。さらに、α-2,6およびα-2,3双方に特異性のある1918ウイルスは感染性が低かった。ヘマグルチニン受容体特異性が、哺乳類におけるインフルエンザ伝播に本質的な役割を果たす。 | 17 |
| 70023 | 2007/04/26 | 70137 | ウイルス感染 | Canadian Blood Services 2006年12月18日 | 2006年12月18日付で、カナダ血液サービスは供血者が供血前に記入する供血記録の問診事項に一部修正を加える。カナダ保健局の指示により、ヒト以外の霊長類(サル、ヒヒ、チンパンジー、アカゲザル、あるいはその血液や唾液)との職業的接触に関する質問を追加した。サル泡沫状ウイルス(SFV)に関する認可された標準検査法がないため、供血者がこの質問に「はい」と答えた場合は無期限に供血延期となる。研究所で霊長類を扱う人、獣医師、動物園職員などが延期対象となるだろう。 | 18 |
| 60247 | 2007/03/29 | 61043 | ウイルス感染 | CDC/MMWR 2007; 56(04): 73-76 | 2006年12月中旬にケニア保健省に発熱と全身出血と伴った原因不明の死亡例数例が北東部のGarissa地区から報告された。12月20日までに計11例の死亡例が報告された。患者19例中10例の血清からリフトバレー熱(RVF)ウイルスRNAまたはRVFウイルスに対するIgM抗体が検出された。黄色熱、エボラ、クリミアーコンゴ出血熱、デングウイルスには全ての血清検体が陰性であった。6検体からRVFウイルスが単離され、確定された。2007年1月25日現在、死亡118例を含む404症例が報告されている。 | 19 |
| 60234 | 2007/03/23 | 61006 | ウイルス感染 | J Infect Dis 2006; 194: 1276-1282 | ヒトボカウイルス感染の疫学的プロフィールおよび臨床的特徴を調べるため、2歳未満の小児のヒトボカウイルスを調査した。直接的免疫蛍光試験でRSV(respiratory syncytial virus)、パラインフルエンザウイルス(1-3型)、インフルエンザAおよびB、並びにアデノウイルスが陰性であった425名中22名(5.2%)がPCRでヒトボカウイルス陽性であり、無症候であった96名では陽性者はゼロであった。この試験期間中、2つの異なる遺伝型が見られた。 | |
| 60248 | 2007/03/30 | 61061 | ウイルス感染 | ProMED-mail20061223.3593 | 日本でノロウイルスによる感染性胃腸炎が増加している。この疾患は従来食中毒とされてきたが、昨年の症例のうち生の貝類摂食に関連したものは15%しかなく、患者の吐瀉物や排泄物から、あるいはウイルスが手を介して食物や食器に付着することで間接的に感染することが多い。今シーズンのノロウイルス流行は主にヒトーヒト感染によるものであり、変異による新たなウイルス株の流行と考えられる。2006年11月27日から12月3日までの間に、全国の約3000の医療機関から65,638人の感染患者が報告された。 | 20 |
| 60248 | 2007/03/30 | 61061 | ウイルス感染 | ProMED-mail20070106.0058 | 2006年12月23日、ケニアGarissaの公立病院に入院した患者複数の症例から、リフトバレー熱のヒトでのアウトブレイクが初めて確認された。IgM及びPCRにより確定診断された。同地区での発病率は、19/10万人で、最高値は最初に患者が見つかったShanta Abakの129/10万人である。2007年1月5日現在で188例に達し、うち68例が死亡した。2007年1月4日、ケニヤ北東部のIjara地区でリフトバレー熱の新規疑い例8例が発見された。 | 21 |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
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| 70023 | 2007/04/26 | 70137 | ウイルス感染 | ProMED-mail20070216.0586 | 西オーストラリア保健当局は、東Kimberleyと東Pilbara地区で蚊が媒介するウイルスの証拠が見つかったとして、西オーストラリア北部に居住あるいは滞在中の人々に、蚊に注意するよう呼びかけた。西オーストラリア大学が実施するサーベイランスプログラムによって、今年の雨期に初めてクンジンウイルスが確認された。クンジンウイルスは、蚊によって媒介されるウイルスで、マレーバレー脳炎(MVE)ウイルスと同じグループに属する。 | 22 |
| 70023 | 2007/04/26 | 70137 | ウイルス感染 | ProMED-mail20070216.0596 | ペルーの地方保健局長官は、Cuzco県La Convencion郡で黄熱による死亡例3例が発生したと報告した。Cuzcoの保健当局によると、このうち1例はMatoriato地区で発生したとのことである。当局は、La Convencion郡に向かう人全員を対象とした黄熱のワクチン接種キャンペーンを含む危機管理計画の策定を決定した。 | 23 |
| 60242 | 2007/03/28 | 61037 | ウイルス感染 | Transfusion 2006; 46: 1352-1359 | 全血輸血により、サルfoamyウイルス(SFV)感染が起こるかをアカゲザルを用いて調べた。自然感染ザル2匹の全血を、各々、レトロウイルスを持たないサル2匹に輸血したところ、1匹のドナーからのレシピエントでは感染し、もう1匹のドナーからのレシピエントは感染しなかった。ヒトでのSFV輸血伝播の可能性が示された。 | |
| 70007 | 2007/04/24 | 70105 | ウイルス感染 | Transfusion 2007; 47: 162-170 | 輸血により、サルfoamyウイルス(SFV)感染が起こるかをアカゲザルを用いて調べた。感染ザルの血液を非感染ザルに輸血したところ、輸血されたサルの血液から8週後にプロウイルスDNAが検出され、その1週間後にセロコンバージョンが起こった。血しょう中に検出限界下限のSFVが検出された。また感染29週目に唾液中にSFVが検出された。輸血によりSFVが感染することが初めて示された。 | 24 |
| 70015 | 2007/04/25 | 70113 | ウエストナイルウイルス | CDC/MMWR 2007; 56(4): 76-79 | ID-NATを用いた強化スクリーニング開始以降に、初めて西ナイルウイルス輸血感染症例が報告された。2006年に免疫不全患者2例が、感染ドナー1例(献血時のMP-NATの結果は陰性)由来の血液製品を投与された後、西ナイル神経侵襲性疾患を発症した。今回の例はID-NATは実施されておらず、ID-NATトリガーを促進することが重要である。 | 25 |
| 60248 | 2007/03/30 | 61061 | ウエストナイルウイルス | ProMED-mail20061214.3510 | 2006年、米国におけるウエストナイルウイルス感染のヒト症例は43州から4052例が報告され、うち1396例で脳炎や髄膜炎を発症、死亡例は146例だった。また、ウマ、トリ、蚊からのウイルス検出が報告されている。 | 26 |
| 60248 | 2007/03/30 | 61061 | ウエストナイルウイルス | Transfusion 2006; 46: 2036-2037 | ウエストナイルウイルス(WNV)が輸血感染するとの認識により、米国とカナダではウイルスRNAに関する供血者の検査が迅速に導入された。最近の分析ではこの検査は費用対効果が低いと指摘されている。Custerらは、ミニプール検査と一部個別検査を組み合わせた通年の検査は、費用対効果は低いが血液安全のためには最善の選択であるとしている。一方Korvesらは、検査の削減を提唱している。検査の効率性を問う必要はあるが、WNVスクリーニングを行う他の方法がないかを検討することも重要である。 | 27 |

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| 60248 | 2007/03/30 | 61061 | クロイツフェルト・ヤコブ病 | Vox Sang 2006; 91(Suppl. 3): 68-69 | 米国で供血後に古典的CJDを発症した個人を特定し、受血者の追跡調査を行った。2006年2月までに、古典的CJDで死亡した供血者31名が試験に登録され、384名の受血者が特定された。追跡を行ったところ、古典的CJDが輸血を介して受血者に伝播したという証拠は示されなかった。本研究は、1995年に米国の大規模血液供給システムと疾病対策予防センター(CDC)により開始され、継続中である。 | |
| 70006 | 2007/04/23 | 70089 | クロストリジウム感染 | YOMIURI ONLINE (2007年2月22日 読売新聞) | 千葉県の船橋市立医療センターは22日、同県内の50歳代の男性が、主に牛の病気の原因とされる「気腫疽菌」に感染し、死亡したことを明らかにした。人への感染が報告されたのは世界初である。気腫疽菌は傷口などから動物の体内に入り、筋肉が壊死する「気腫疽」を発症させる。同センターは、「気腫疽菌は人には感染しないというのがこれまでの常識だった。詳しい感染経路を調べることが今後の課題」としている。 | 28 |
| 70003 | 2007/04/13 | 70055 | クロストリジウム感染 | イザ(産経新聞) 2007年2月23日 | 千葉県の船橋市立医療センターは22日、2006年2月に搬送され、死亡した同県内の50歳代の男性から気腫疽菌が検出されたと2007年2月22日に発表した。人への感染が報告されたのは初めてである。 | 29 |
| 60248 | 2007/03/30 | 61061 | チクングニヤウイルス感染 | Eurosurveillance 2006; 11(8): 060810 | 2005年12月以降、チクングンヤウイルス感染のアウトブレイクがインドの8つの州で続いており、拡大するおそれがある。最も被害の大きい5つの州では896500例以上の疑い例が報告されている。北部の州からは1例も報告されていない。ヨーロッパの多数の国で輸入症例が報告されている。感染の拡大防止ならびに特異的な抗ウイルス薬とワクチンの開発が急務である。 | 30 |
| 60248 | 2007/03/30 | 61061 | チクングニヤウイルス感染 | 毎日新聞 2007年1月24日 | 1月24日、厚生労働省はスリランカから帰国した30歳代の女性が、チクングニヤ熱に感染していたと発表した。国内で日本人の感染が確認されたのは初めてである。女性は2006年11月中旬、スリランカで発熱し、現地でチクングニヤ熱かデング熱と診断された。女性はすでに症状は回復し、在住するスリランカに戻っている。厚労省によると、チクングニヤ熱は発熱や関節炎、発疹などが特徴で、死亡率は極めて低い。蚊を介して感染し、人から人への感染はない。 | 31 |
| 60248 | 2007/03/30 | 61061 | トリパノソーマ症 | AABB Weekly Report 2006; 12(43): 1-2 | FDAがシャーガス病の供血者スクリーニング検査試薬を初めて認可したのを受け、米国血液銀行協会(AABB)は、採血施設が検査導入とその期間を決定し、供血者と受血者のフォローアップのためのガイダンスを提供するのに役立つよう、協会公報#06-08を12月14日に発表した。具体的な勧告内容は、出荷停止、遡及調査、自己血輸血で繰り返し検査陽性となった場合の成分製剤出荷の認可、供血延期措置、通知、確認試験、供血者の医学的評価のための供血延期などの事項が盛り込まれている。 | 3( |
| 60248 | 2007/03/30 | 61061 | トリパノソーマ症 | CDC/MMWR 2006; 55(29); 798-800 | ロサンジェルスの心臓移植患者2名で、臓器移植によるシャーガス病伝播が見られた。1例は拒絶反応による合併症で死亡し、もう1例は心不全で死亡した。両例ともドナーがTrypanosoma cruzi感染者であったことが明らかとなった。米国における固形臓器移植によるT. cruzi伝播としては4例目と5例目になる。 | |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
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| 60234 | 2007/03/23 | 61006 | トリパノソーマ症 | FDA News P06-198 2006年12月13日 | 米国FDAは2006年12月13日、重篤且つ致死性の寄生虫感染症のシャガス病を引き起こす血液寄生虫について血液ドナーをスクリーニングする新しい検査を承認した。この試験はORTHO T. cruzi ELISA Test Systemと呼ばれ、trypanosoma cruzi抗体を検出するもので、このような検査では初めてFDAに承認されたものである。この検査は、全血の供血者のスクリーニングに加えて、臓器、細胞及び組織ドナーからの血漿及び血清をスクリーニングするのに用いられる。 | 33 |
| 70034 | 2007/04/27 | 70150 | トリパノソーマ症 | Transfusion 2007; 47: 540-544 | 神経芽細胞腫(ステージ4)を発症した3歳半の女児が複数の血液成分製剤投与を受けた後、Trypanosoma cruziによるシャーガス病と診断された。輸血された製剤の全供血者の血液を再検査したところ、初回供血者1名がT. cruzi抗体陽性であることが判明した。当該供血者は、ボリビア出身であり、17年前に米国に移住した。移住後は母国に帰国していない。本症例は、米国・カナダでの輸血によるシャーガス病感染の7例目の報告である。シャーガス病スクリーニング検査が必要であることを示している。 | 34 |
| 60248 | 2007/03/30 | 61061 | トリパノソーマ症 | Vox Sang 2006; 91(Suppl 3): 98 | フランスでリスクのある供血者に対し、シャーガス病スクリーニング検査を行うこととなり、Bioelisa Chagas、Chagatek、Ortho T.cruzi ELISA Test System ' 1、ELISA Cruzi の4種類のTrypanaosoma cruzi ELISAキットの感受性、再現性、特異性を評価した。感受性に関しては4種類の検査法で正確に検出された。再現性は24コピーのS/CO値の平均の変動係数によって示され、各々、6.30%、9.30%、15.7%、22.40%だった。 | |
| 60248 | 2007/03/30 | 61061 | パルボウィルス | Transfusion 2006; 46:1593-1600 | パルボウイルスB19ジェノタイプ3流行地域であるガーナで、114組の成人供血者-小児受血者ペアにおける輸血によるB19の伝播および免疫学的特性について調べた。2例の供血がパルボウイルスB19DNAと特異的抗体を含有していたが、伝播は見られなかった。B19 IgG含有の全血がB19DNA陽性受血者14例に輸血されていたが、その内、輸血前にVP2に対する抗体が認められなかった10例ではウイルスが除去された。 | |
| 60248 | 2007/03/30 | 61061 | マラリア | AABB Weekly Report 2006年9月15日 | 2006年9月8日付けのKorea Timesによると、交通事故後に輸血を受け、その後マラリアと診断された患者が、疑わしい血液の流通を防ぐことができなかった韓国赤十字を批判している。韓国赤十字は状況を知りながら、何の行動も起こさず、その結果、不適切な血液製品が流通し続けたとのことである。輸血によるマラリア感染で既に1名の患者が死亡している。 | |
| 60237 | 2007/03/27 | 61024 | マラリア | AABB Weekly Report 2006; 12(32): 10 | 2006年9月8日付けのKorea Timesによると、交通事故後に輸血を受け、その後マラリアと診断された患者が、疑わしい血液の流通を防ぐことができなかった韓国赤十字を批判している。韓国赤十字は状況を知りながら、何の行動も起こさず、その結果、不適切な血液製品が流通し続けたとのことである。輸血によるマラリア感染で既に1名の患者が死亡している。 | |
| 60248 | 2007/03/30 | 61061 | マラリア | CDC/MMWR 2006; 55(37): 1013-1016 | 2006年9月19日、CDCは米国在住のグレートエグズーマ島渡航者向けの抗マラリア薬服用勧告を解除した。2006年5月～6月の間に合計19例のマラリア症例が確認されたが、3ヶ月間新規症例の報告がなかったためである。 | |
| 60248 | 2007/03/30 | 61061 | マラリア | Eurosurveillance 2006年11月16日 | 2006年8月にコルシカ島で三日熱マラリア1症例が診断された。フランス南東部出身の59歳男性で、2006年夏に南コルシカのポルトに滞在していた。患者はマラリア流行地域への渡航歴はなかった。マダガスカルに渡航歴のある三日熱マラリア患者が7月初めに同地区に滞在しており、コルシカのハマダラカによってP. vivaxの国内伝播が起こったことを示唆している。本症例は、この地域で報告されたマラリアの地域内伝播の1972年以来初の症例である。 | 35 |

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| 60248 | 2007/03/30 | 61061 | マラリア | Eurosurveillance weekly release 2007; 12(1): 070111 | 輸入感染症サーベイランスに関するヨーロッパネットワークへの報告数によると、2006年11月下旬以降、インド、ゴア州への渡航者において、熱帯熱マラリア患者が増加している。1月10日までに、ドイツで2例、デンマークで4例、スエーデンで2例、計8例の患者が報告された。 | 36 |
| 60248 | 2007/03/30 | 61061 | マラリア | ProMED-mail20061004.2843 | 中国東部の安徽省でマラリア感染が拡大している。地元の保健当局によると、今年に入ってから2006年9月25日時点で17917例が報告されており、昨年の同時期と比較すると89.8%増加している。保健当局は緊急通告を出して、感染の監視を強化し、拡大防止のためにマラリア予防と治療に関する啓発を行うよう地元自治体に要請した。 | |
| 70048 | 2007/05/22 | 70198 | レンサ球菌感染 | ProMED-mail20070223.0668 | 米国の科学者は北アメリカで初めて報告されたStreptococcus suis髄膜炎のヒト感染例を確認した。健康であった59歳の男性農業従事者が髄膜炎で入院し、S. suis感染と判明した。S. suisはブタで重病を起こすグラム陽性球菌であり、ブタを扱う職業の人は注意が必要である。保健当局はヒトからヒトへの感染のおそれはないとしている。 | 37 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | AABB Weekly Report 2006; 12(44): 4-5 | 伝達性海綿状脳症(TSE)諮問委員会が2006年12月15日に公開で開催され、ヒト血漿由来抗血友病因子(FVIII)製剤におけるvCJDへの潜在的曝露に関するFDAのリスク評価ならびに血漿由来FVIII製造におけるTSEクリアランスのレベルについて討論された。このリスク評価に対して諮問委員会は、報告が強制でないことや、最終製品のリスク減少を推定をする際に用いたエビデンスに対して懸念を表明した。 | 38 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | AABB Weekly Report 2006年7月21日 | アイルランド輸血サービスは、CJDの病因となるプリオンを供血血液から除去するために開発された新しい装置を1年間使用した後、試用の中止を決定した。血液サービスは昨年、そのフィルター装置を購入したが、十分な効果が得られず、CJDプリオンは捕捉されずに通過し、供血中に混入する可能性があるためである。 | |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | ABC Newsletter 2006 Sep 1; 16 | 外科用器具には殺菌後も依然としてクロイツフェルトヤコブ病(CJD)が残存する可能性が明らかとなった。エジンバラ大学研究チームは、タンパク質による平均汚染量が器具1mm2あたり0.2μgであり、ヒトへの感染に必要な量をかなり上回る数値であることを認めた。最大量は、扁桃腺(プリオンが分布することが知られている組織のひとつ)の切除に用いた器具で認められた。同チームは、保健省に対し、同チームが開発したガスプラズマ滅菌の広範な導入を推奨している。 | |
| 60255 | 2007/03/30 | 61068 | 異型クロイツフェルト・ヤコブ病 | ABC Newsletter 2006年12月15日 5-6ページ | 米国で製造された血漿由来の第Ⅷ因子製剤による患者へのvCJD病原体伝播のリスクは、極めて低いと見られる。生物製剤評価調査センター(CBER)のSteven Anderson博士は、「しかし、リスクはゼロではない」と伝達性海綿状脳症(TSE)諮問委員会の本日の会合で話した。CBERは、2005年10月31日の委員会で提示されたコンピュータモデルと仮説に基づいたリスク分析案の概要を示した。重要度解析では、リスクを決定する主要な要素は、製造工程におけるvCJD感染因子の低減である。 | 39 |
| 70023 | 2007/04/26 | 70137 | 異型クロイツフェルト・ヤコブ病 | ABC Newsletter 2007年2月9日 7-8ページ | 将来のvCJDによる死亡率は、供血に関する公衆衛生上の施策によって予想されていたよりも遙かに低くなるだろうと英国の研究者が報告した。Royal Society Journal Interface誌オンライン版によると、2080年までの輸血によるvCJDの死亡例は50例と予測される。感染牛の摂食によるvCJD感染が排除されたため、現在では輸血による伝播が最も可能性が高いと研究者は話している。 | 40 |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
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| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | CDC 2006年11月29日 | 米国で3例目のvCJD症例が確定された。サウジアラビアで生まれ育った若年成人で、2005年後半から米国に住んでいる。2006年11月下旬にアデノイドおよび脳生検により確定診断された。この患者に輸血歴やヨーロッパ訪問歴はなく、子供の頃にサウジアラビアでBSE感染牛製品を摂食したことが原因と思われる。この患者に供血歴はなく、公衆衛生学的調査により、米国住民への伝播の危険はないと同定された。 | 41 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | FDA/CBER 2006年10月15日 FDA/TSEAC Meeting 2006年12月15日 | FDAは、米国で認可されたヒト血漿由来第VIII凝固因子製剤(pdFVIII)の使用に係る潜在的vCJDリスク評価草案を作成した。FDAの評価モデルの結果は、血友病Aおよびフォンウィルブランド病患者に使用されるpdFVIII製剤の、vCJD感染リスクは非常に低いが、ゼロではないかもしれないことを示唆した。またTSEAC(TSE Advisory Committee)は、pdFVIII製品中のTSE除去の適切な閾値について議論した。TSE除去レベルにより、vCJD感染リスクは大きく変動することが示された。 | |
| 70027 | 2007/04/26 | 70141 | 異型クロイツフェルト・ヤコブ病 | FDA/CBER 2006年11月27日 | FDAは、米国で認可されたヒト血漿由来第VIII凝固因子製剤(pdFVIII)の使用に係る潜在的vCJDリスク評価草案を作成した。FDAの評価モデルの結果は、血友病Aおよびフォンウィルブランド病患者に使用されるpdFVIII製剤の、vCJD感染リスクは非常に低いが、ゼロではないかもしれないことを示唆した。製造工程での原因物質除去レベルにより、vCJD感染リスクは大きく変動する。 | 42 |
| 70055 | 2007/05/24 | 70213 | 異型クロイツフェルト・ヤコブ病 | FDA/CBER 2007年3月15日 | FDA、CDCおよびNIHを含む米国Public Health Serviceは、米国で承認された血漿由来第VIII因子製品を投与された血友病AおよびフォンウィルブランドVCJDリスクは極めて小さいとの見解を示した。血漿由来第IX因子を含む他の血漿由来製品によるvCJDリスクは同程度もしくは更に小さいと思われる。 | 43 |
| 70006 | 2007/04/23 | 70089 | 異型クロイツフェルト・ヤコブ病 | FDA/TSEAC Meeting 2006年12月15日 | FDAは、米国で認可されたヒト血漿由来第VIII凝固因子製剤(pdFVIII)の使用に係る潜在的vCJDリスク評価草案を作成した。FDAの評価モデルの結果は、血友病AおよびフォンウィルブランドVCJDリスクは非常に低いが、ゼロではないかもしれないことを示唆した。またTSEAC(TSE Advisory Committee)は、pdFVIII製品中のTSE除去の適切な閾値について議論した。TSE除去レベルにより、vCJD感染リスクは大きく変動することが示された。 | 44 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | Future Virol 2006; 1: 659-674 | 血液製剤の製造工程におけるプリオンの除去に関する総説である。プリオン除去のための個々の製造工程は、実際の製造条件を実験室での条件にスケールダウンさせ、確立されているスクレイピー株をモデル系として用いて通常は評価されている。しかしながら、血液中のプリオンタンパクの存在形態が不明なので、評価実験のためのスパイク材料としてのプリオンの調製方法は注意深く考慮しなければならない。現在のところ、エタノール分画、PEG 分画、カラムクロマトグラフィー、ウイルス除去膜およびデプスフィルターでの濾過が有効とされている。 | |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | Guidance for Industry (DRAFT GUIDANCE) FDA/CBER August 2006 | 古典的CJDの潜伏期間は38.5年であり、vCJDの潜伏期間も非常に長いことが示唆されている。また、未確認ではあるが恐らくかなりの数の血液ドナーが、欧州におけるBSE激増中にフランスで感染した可能性がある。これらのことから、FDAは1980年以降フランスで血液又は血液成分の輸血を受けた者からの供血を無期限に停止するという予防策の導入をガイダンス案として発表した。 | |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | Health Protection Report 1(3) 2007年1月19日 | 英国で4例目の輸血関連vCJD可能性例が診断された。この症例は供血後約17ヶ月でvCJDを発症したドナーからの赤血球輸血を受け、8年半後にvCJDを呈した。このドナーは3例目の輸血関連vCJD症例へのドナーでもある。4例目の症例はプリオン蛋白遺伝子のコドン129がメチオニンホモ体であった。まだ生存中である。 | 45 |

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| 70006 | 2007/04/23 | 70089 | 異型クロイツフェルト・ヤコブ病 | J R Soc Interface doi:10.1098/rsif.2007.0216 Published online | 血液由来のvCJDの流行の大きさを探るために感度分析を行い、公衆衛生的介入の有効性について調査した。数学的モデルを開発し、悲観的モデリング仮定で評価すると、自己持続的流行が起こるならば2080年までに900例以内、楽観的仮定では250例以内となった。大規模な又は自己持続性流行に至るシナリオの可能性はあるが実現性は低く、輸血を受けたヒトからのドネーション禁止措置等の公衆衛生的介入が有効である。 | 46 |
| 70021 | 2007/04/25 | 70125 | 異型クロイツフェルト・ヤコブ病 | J Virol 2006; 80: 9104-9114 | ヘラジカのプリオン(ElkPrP)またはシカのプリオン(DePrP)を発現するトランスジェニック(Tg)マウスを作成したところ、600日齢以上でも自発的な神経学的異常は示さなかった。これらのマウスに慢性消耗病(CWD)陽性のヘラジカまたはシカの脳検体を接種したところ、Tg(ElkPrP)マウスでは180-200日後に、Tg(DePrP)マウスでは300-400日後に、発病した。発病したマウスの脳にはPrPアミロイドプラークが多数見られた。ヒト、ウシまたはヒツジPrPを過剰発現したTgマウスはCWDプリオン接種後500日以上経っても発病しなかった。 | 47 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | Lancet 2006; 368: 2061-2067 | vCJDを発症した供血者の輸血を受けた患者が神経学的徴候を発現し、National Prion Clinicへ照会され、vCJDと診断された後、MRC PRION-1 trialに登録された。患者が死亡した際、剖検時に脳と扁桃腺の組織を得、免疫ブロッティング法および免疫組織化学検査により異常プリオンの存在を調べた。剖検により診断が確認され、扁桃腺のプリオン感染が示された。扁桃腺の生検は、BSEプリオンの1次感染患者と同様、医原的曝露を被った他の高リスク患者においても、早期の症状発現前診断を可能にする。 | 48 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | LANCET 2006; 368: 2226-2230 | ヒト濃縮赤血球に混入した脳由来の感染性物質を約4 log ID50減らすことのできるアフィニティ樹脂L13と同等能力のL13Aとについて、血中に存在する内因性TSE感染性物質の除去能力を評価した。スクレイピーに感染させたハムスターの全血は白血球除去によって感染性の72%が除去された。99匹中15匹が白血球除去した全血に感染したが、更に各々の樹脂を通過して得られた最終産物を接種された96匹又は100匹はいずれも発症しなかった。樹脂によって内因性TSE感染性物質が除去されることが示された。 | 49 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | PLoS ONE 2006; 1: e71 | プリオン蛋白に高親和的、特異的に結合する吸着基質Alicon Prio Trapを開発し、ヒト、ウシ、ヒツジ、ヤギの乳汁中にPrPScの前駆体であるPrPCを同定することができた。PrPCの絶対量には種差があり、ヒツジの乳汁中でμg/mlレンジ、ヒト乳汁中ではng/mlレンジであった。PrPCは、均質化し低温殺菌した市販ミルク中にも認められ、超高温処理を施しても内因性PrPC濃度はわずかに減少しただけであった。TSEに感染した動物の乳汁がPrPScの感染源となる可能性を示唆する。 | 50 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | PLoS Pathogens 2006; 2: 956-963 | 最近、大規模なスクリーニングによって、従来とは異なるPrPresがウシにおいて発見された。H型と呼ばれる高分子量のフランスのウシPrPres分離株を、ウシまたはヒツジのPrPを発現するトランスジェニックマウスに接種した。全てのマウスは神経学的症状を呈し、死亡し、これらの株が感染性プリオンの新規株であることが示された。この病原体は、BSE病原体およびヒツジスクレイピー病原体とは明らかに異なる特有の神経病理学的特徴を示した。 | |

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| 70023 | 2007/04/26 | 70137 | 異型クロイツフェルト・ヤコブ病 | Proc Natl Acad Sci USA 2007; 104: 1965-1970 | スクレイピー22L株に感染した神経芽細胞腫細胞およびFUクロイツフェルトヤコブ病病原体に感染した視床下部GT細胞は直交配列で高密度な25nmウイルス様粒子を示した。この粒子は膜に囲まれた不完全結晶で、A型レトロウイルス粒子クラスターや異常PrP原線維とは別に存在し、形態学的にも異なっていた。またPrP抗体でラベルされず、ホルボールエステル処理で増加しなかったことから、プリオンではなかった。この粒子は後期PrP脳病変を誘発するTSE原因ビリオンである可能性がある。 | 51 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | ProMED-mail20070108.0081 | 英国保健省は2007年1月8日、CJD患者数に関する最新情報を公表した。vCJD確定例における死亡患者112名、vCJD可能性例における死亡患者(神経病理学的に未確定)46名で、死亡患者総数は158名である。生存中のvCJD可能性患者は7名で、vCJD確定例または可能性例総数は165名である。2006年12月4日の月例統計以来、死亡患者総数には変化なく、確定例または可能性例総数は1名増加した。このデータは英国におけるvCJD流行は減少しつつあるとする見解に一致する。 | 52 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | Science 2006; 314: 133-136 | 慢性消耗病(CWD)非感染シカをCWD陽性のシカの唾液、血液または尿・糞に曝露させた。その結果、CWDを伝播しうる感染性プリオンが唾液および血液中に認められた。CWDはシカ科の動物に容易に伝播すると言える。プリオン感染では体液との接触に関する注意が払われるべきである。 | |
| 70021 | 2007/04/25 | 70125 | 異型クロイツフェルト・ヤコブ病 | Vaccine (2006), doi:10.1016/j.vaccine.2006.10.058 | ヒトや動物におけるTSEの多様性を解明するため、PrPresの詳細な特徴が研究されている。分子学的な方法により、最近、ヨーロッパと米国の感染牛で異常なBSE型が発見され、少なくともBSE牛の何例かには別の起源の可能性が出てきた。小型反芻動物での新しいTSE型は「非典型的スクレイピー」または「Nor98」と呼ばれ、ヨーロッパの大部分の国でTSE迅速検査で同定されている。 | 53 |
| 70021 | 2007/04/25 | 70125 | 異型クロイツフェルト・ヤコブ病 | Vet Rec 2007; 160: 215-218 | カナダで乳牛のBSE感染が確定したことで、牛肉だけでなく牛乳や乳製品が病原体プリオンを含有しているかが大衆の関心事となった。このレビューは、牛乳や初乳中のプリオン、ならびに種々の動物系における垂直および水平感染に関する研究から牛乳の安全性に関するエビデンスを検討した。エビデンスは牛乳の消費による新たなvCJD感染のリスクは無視できることを示した。 | 54 |
| 60248 | 2007/03/30 | 61061 | 異型クロイツフェルト・ヤコブ病 | Vox Sanguinis 2006; 91(Suppl 3): 70 | PRDT(Pathogen Removal and Diagnostics Technologies)は、全血、RBCまたは血漿存在下で,脳由来プリオンタンパク質およびTSE感染物と強く結合する高親和性リガンドを得るため、何百万もの化合物をスクリーニングした。その結果、PRDTのリード樹脂は赤血球存在下でも高濃度のTSE感染物を吸着し、低濃度の内因性TSE感染物を除去した。この樹脂を使用したMacoPharma P-Capt(TM)フィルターを用いることにより、輸血によるvCJD伝播リスクを軽減できる。 | |
| 70017 | 2007/04/25 | 70121 | 異型クロイツフェルト・ヤコブ病 | Vox Sanguinis 2006; 91: 221-230 | 散在性(s)、家族性(f)および異型(v)クロイツフェルト・ヤコブ病(CJD)が輸血を介して感染伝播するかを判定するために、英国CJDサーベイランスユニットと血液サービスにより進行中の試験の2006年3月1日までの結果である。供血者として報告されたvCJD 31例中18例、sCJD 93例中3例およびfCJD 5例中3例の血液が、各々、66例、20例および11例の受血者へ輸血されていた。2例の受血者においてvCDJが確定していた。また、vCJD11例中7例、sCJD52例中7例が、輸血の既往歴があった。 | 55 |

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| 70017 | 2007/04/25 | 70121 | 異型クロイツフェルト・ヤコブ病 | Vox Sanguinis 2007; 92: 121-124 | ハプトグロビンおよび抗トロンビンの2つの異なった調整液にヒトパルボウイルスB19を加え、60℃で10時間処理した。異なった溶液中のB19は加熱中異なった熱感受性パターンを示し、ハプトグロビン調整液中では緩やかな不活性化、抗トロンビン調整液中では限定的な不活性化であった。異なった調整液を用いた以前の研究ではB19は迅速に不活性化され、今回の不活性化の動力学とは大きく異なった。B19の熱感受性は溶液組成に大きく依存する。 | 56 |
| 60234 | 2007/03/23 | 61006 | 異型クロイツフェルト・ヤコブ病 | 英国保健省 Press Statement 2007年1月18日 | 輸血と関係した新たなvCJD疾患(4例目)が、最近診断された。この症例は後にvCJDを発症したドナーからの輸血を受けてから約9年後にvCJDと診断された。同じ供血者からの輸血は以前に同定された1例とも関係していた。4例目の患者は以前からvCJDに暴露した可能性を知らされていた。4例目のvCJD感染症例により、輸血を介したヒトの間におけるvCJD感染リスクについての懸念が高まっている。4症例は全て、成分輸血に関係したものであり、血漿分画製剤による治療に関連した症例は今まで報告されていない。 | 57 |
| 60237 | 2007/03/27 | 61024 | 異型クロイツフェルト・ヤコブ病 | 英国保健省 Press Statement 2007年1月18日 | 英国で輸血と関係した新たなvCJD症例(4例目)が、最近診断された。この症例は後にvCJDを発症したドナーからの輸血を受けた約9年後にvCJDと診断された。同じ供血者からの輸血は以前に同定されたvCJD1例とも関係していた。4例目の患者は以前からvCJDに暴露した可能性を知らされていた。4例目のvCJD感染症例により、輸血を介したヒトの間におけるvCJD感染リスクについての懸念が高まっている。4症例は全て、成分輸血に関係したものであり、血漿分画製剤による治療に関連した症例は今まで報告されていない。 | 58 |
| 60237 | 2007/03/27 | 61024 | 異型クロイツフェルト・ヤコブ病 | 厚生労働省 平成18年8月24日 | 平成18年8月23日に開催された薬事・食品衛生審議会血液事業部会安全技術調査会において、ヒト胎盤エキス(プラセンタ)注射剤使用者に対する献血制限措置を日本赤十字社が実施することが了承された。 | |
| 60248 | 2007/03/30 | 61061 | 感染 | Blood 2006; 108: Abstract #4144 | 0.2mM S-303 (アクリジン化合物) および20mM グルタチオン (GSH) を用いた改良S-303処理法を用い、RBC中の細菌およびウイルス不活化の有効性を評価した。輸血に関連するグラム陽性菌およびグラム陰性菌、Vesicular stomatisウイルス、Adenovirus 5、HIVおよびウシウイルス性下痢ウイルス(HCVのモデル)のいずれも改良S-303処理により効果的に不活化された。 | 59 |
| 60248 | 2007/03/30 | 61061 | 狂犬病 | ProMED-mail20061118.3303 | 2006年11月17日、京都府の保健所は、京都市の60歳代の男性がフィリピンで犬にかまれ、帰国後に狂犬病を発症して死亡したと発表した。厚労省によると、日本人が国内で狂犬病を発症したのは36年ぶりである。厚労省によると、男性はフィリピン滞在中の8月末に野良犬にかまれ、11月1日に帰国した。9日に風邪のような症状で京都市内の病院を受診した。その後、幻覚症状、水や風を怖がるなど狂犬病特有の症状を発症した。国立感染症研究所が調べたところ、男性の唾液から狂犬病ウイルスが検出された。 | 60 |
| 60234 | 2007/03/23 | 61006 | 結核 | NIKKEI NET いきいき健康 2006年12月5日 | 既存の治療薬がほとんど効かず、世界保健機関(WHO)が警戒を呼び掛けている「超多剤耐性」の結核菌が、国内でも入院患者の0.5%から検出されたことが、結核研究所の調査で明らかになった。2002年6月から11月にかけて国内99の結核治療施設の入院患者3122人から採取した結核菌を分析した結果である。検出例の半数は薬の服薬歴がなかったことから、他の患者から感染した可能性が高い。 | 61 |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
|---|---|---|---|---|---|---|
| 60241 | 2007/03/28 | 61036 | 細菌感染 | Epidemiol Infect 2006; 134: 1068-1073 | Mycobacterium bovisはニュージーランドの野生動物や家畜を宿主とする。1995-2002年のヒト結核症例を疫学的、臨床検査学的に調べた結果、確定症例1997例中54例(2.7%)がM.bovisで、10万人当たり0.2人の割合であった。ヒトからの単離体23例中14例がウシ、シカ、オポッサム、フェレット、ブタ、ネコからの単離体と同一の制限酵素分析パターンを示した。ニュージーランドでは動物宿主からヒトへの伝播が低レベルで続いていることが示唆された。 | 62 |
| 70017 | 2007/04/25 | 70121 | 重症急性呼吸器症候群 | Transfusion 2006; 46: 1770-1777 | 血液由来製品を模した蛋白質溶液中の重症急性呼吸器症候群コロナウイルス(SARS-CoV)を不活性化する方法として、加熱、UV照射、オクタン酸、溶剤/界面活性剤(S/D)法を検討した。その結果、60℃で15-30分間加熱および40分間UVC照射はSARS-CoVを不活性化した。UVA照射はソラーレン添加を必要とし、オクタン酸処理はSARS-CoVを不活性化できなかった。S/D処理は、SARS-CoV不活性化に、Triton X-100は2時間、Tween 80は4時間、石炭酸ナトリウムは24時間を要した。 | 63 |
| 60248 | 2007/03/30 | 61061 | 鳥インフルエンザ | Eurosurveillance 2006; 11(12): 061221 | 2006年11月29日時点でH5N1型トリインフルエンザウイルス感染患者258名がWHOに報告され、50カ国以上で鳥類での感染が確認されており、うち10カ国では鳥類がヒト患者発生の感染源となっている。EUでは、同ウイルスは家禽には感染定着しておらず、2006年春季に少なくとも15カ国で野鳥の感染が確認されたが、ヒト感染症例は発生していない。家禽の感染予防が成功し、感染は5件のみで迅速に制圧された。散発例の報告が続いていることから、生物学的安全確保対策と早期警報システムを堅持する必要がある。 | 64 |
| 60248 | 2007/03/30 | 61061 | 鳥インフルエンザ | ProMED-mail20061201.3394 | WHOは、H5N1鳥インフルエンザウイルスにより光を当て、パンデミック株への変異の検出を容易にするために、H5N1鳥インフルエンザのヒト症例調査のためのガイドラインを発表した。14ページのガイドラインは、患者の問診、周辺で他の症例を捜索することによる接触歴の調査、ヒトーヒト感染の何らかの徴候を発見するためのデータのふるいわけなど、各症例の徹底的な調査を求めている。ガイドラインでは、臨床検査の結果が出る前に疑い症例の調査を行うことを要請している。 | 65 |
| 60248 | 2007/03/30 | 61061 | 鳥インフルエンザ | ProMED-mail20070120.0260 | 2007年1月18日、農林水産省は、宮崎県の養鶏場で発生したトリインフルエンザは高病原性ウイルスによるものだったと明らかにした。同省は養鶏場で死亡した鶏から採取したウイルスのサンプルを検査して病原性が高いものであることを確認した。H5N1型ウイルスの流行は、宮崎県清武町の谷口孵卵場黒坂農場で発生し、3つある鶏舎のうち1つで3500羽の鶏が死亡した。 | 66 |
| 70006 | 2007/04/23 | 70089 | 鳥インフルエンザ | Transfusion 2007; 47: 452-459 | 血漿製剤の製造中に通常使われるウイルス不活性化処理、即ち、ヒトアルブミンに対するパスツール法、静注用免疫グロブリン(IVIG)に対するSD処理、第VIII因子インヒビターバイパス活性に対する蒸気加熱、及びIVIGに対する低pHインキュベーションが、H5N1インフルエンザウイルス不活化に有効かを再集合体株を使って調べた。その結果、H5N1インフルエンザウイルスは、エンベロープウイルスと同様の挙動を示し、これらのウイルス不活化処理によって効果的に不活化された。 | 67 |
| 60234 | 2007/03/23 | 61006 | 梅毒 | Lancet 2007; 369: 132-138 | 中国の性感染症サーベイランスシステム及び監視サイトネットワークからの症例報告データを収集し評価した。中国における報告された梅毒の全症例発生率は、1993年には100,000人あたり0.2例であったが、2005年には、第一期及び第二期梅毒だけで100,000人あたり5.7例であった。先天的な梅毒の発生率は、1991年は100,000例の出生児あたり0.01症例であったが、2005年には100,000例の出生児あたり19.68症例まで、年平均71.9%の割合で大きく増加した。 | 68 |

| 血対ID | 受理日 | 番号 | 感染症(PT) | 出典 | 概要 | |
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# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2006. 12. 26 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | Tjon GM, Coutinho RA, van den Hoek A, Esman S, Wijkmans CJ, Hoebe CJ, Wolters B, Swaan C, Geskus RB, Dukers N, Bruisten SM. J Med Virol. 2006 Nov;78(11):1398-405. | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | オランダ |

**研究報告の概要**

○免疫正常者におけるA型肝炎ウイルスの多量かつ持続的な排泄

A型肝炎ウイルス(HAV)感染患者の血液および糞便中へのウイルス排泄期間および排泄量を、アラニンアミノトランスフェラーゼ(ALT)、疾患重症度、HAV遺伝子型との関連において調べた。急性A型肝炎患者27名(年齢の中央値:33歳)から臨床データ、血液、糞便を、最長26週間収集した。別の急性A型肝炎患者55名から得た単回輸血検体(single blood donations)も使用した。ウイルス量は、競合的nested RT-PCR法を用いて定量した。HAVは、疾患発症後から81日間(中央値)便中に排泄され、50%の患者で36日目においても多量なウイルスの排泄が続いた(懸濁液1mL当たり$2\times10^{6}$～$2\times10^{8}$コピー)。ウイルス血症が検知されたが、ウイルス量は検出限界以下であり、ウイルス血症期間の中央値は発症後42日間であった。疾患発症後10日間は、ALT値の高さは血中ウイルス量の高さに相関した。遺伝子型1aと1bの患者を比較したところ、HAV排泄期間および黄疸の期間に有意差はなかった。結論として、発症後最初の1ヶ月間は便中に排泄されるHAVの力価が高く、このため患者が感染性を持つ期間はおそらく現在考えられているより長いであろう。血液銀行は、ウイルス血症が1ヶ月以上存続すること、ならびにウイルスの排泄や黄疸の期間に遺伝子型が影響しないことを認識する必要がある。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 免疫正常者において、A型肝炎発症後から81日間(中央値)便中にHAVが排泄され、最初の1ヶ月間は便中に排泄されるHAVの力価が高く、ウイルス血症期間の中央値は発症後42日間であったとの報告である。 | 日本赤十字社は、輸血感染症対策として、問診で肝炎の既往があった場合、A型肝炎については治癒後6ヶ月間、家族に発症した人がいる場合は1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(1)

Journal of Medical Virology 78:1398–1405 (2006)

# High and Persistent Excretion of Hepatitis A Virus in Immunocompetent Patients

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The duration and level of virus excretion in blood and faeces of patients with hepatitis A virus (HAV) infection were studied in relation to levels of alanine aminotransferase (ALT), disease severity and HAV genotype. Clinical data, blood and faeces were collected from 27 patients with acute hepatitis A (median age: 33 years) for a maximum of 26 weeks. Single blood donations from 55 other patients with acute HAV (median age: 32 years) were also used. Virus loads were quantified by competitive nested RT-PCR. HAV was excreted in faeces for a median period of 81 days after disease onset, with 50% of patients still excreting high levels at Day 36 ($2 \times 10^6$–$2 \times 10^8$ copies/ml faeces suspension). Viraemia was detected, but not quantifiable, for a median period of 42 days. In the first 10 days of illness, higher ALT levels were correlated with higher viraemia levels. Comparison of patients infected with genotype 1a with those infected with type 1b did not differ significantly in terms of the duration of HAV excretion or jaundice. In conclusion, faecal excretion of HAV is at a high titre in the first month, perhaps making patients infectious for a longer period than assumed currently. Blood banks should be aware that viraemia may be present for more than 1 month, and genotype did not affect the duration of virus excretion or jaundice. ***J. Med. Virol. 78:1398–1405, 2006.*** © 2006 Wiley-Liss, Inc.

**KEY WORDS:** HAV excretion; HAV viraemia; HAV levels

## INTRODUCTION

Hepatitis A virus (HAV) belongs to the *Picornaviridae* family and is classified in its own genus, *Hepatovirus*. Infection takes place mainly via the faecal-oral route through person-to-person contact. In the late incubation period and at onset of illness very large numbers of HAV particles are excreted in faeces until alanine aminotransferase (ALT) reaches peak elevation [Ciocca, 2000]. Therefore, patients with HAV are considered contagious from 1–2 weeks before onset of symptoms until 1–2 weeks afterward [Koff, 1998; Center for Disease Control and Prevention, 2004]. However, faecal excretion can last up to 3 months and longer [Yotsuyanagi, 1996; Costa-Mattioli et al., 2002a], indicating that patients may be contagious for a longer period than assumed previously.

In addition to faecal excretion, the virus is also present in blood [Center for Disease Control and Prevention, 2004]. HAV viraemia can exceed 3 months in immunocompetent patients [Bower et al., 2000;

Reporting and interviewing of hepatitis A cases were done at the Dutch Public Health Services of Amsterdam, Brabant, South Limburg, Groningen, South-Holland South and South-Holland North. HAV RNA isolation, detection, quantification and sequencing were performed at the Public Health Service of Amsterdam, The Netherlands.

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Costa-Mattioli et al., 2002b; Sagnelli et al., 2003; Normann et al., 2004] and in one HIV-infected patient, it was detected for 256 days [Costa-Mattioli et al., 2002a]. Parenteral transmission has been reported by blood transfusion and blood products [Mannucci et al., 1994; Soucie et al., 1998] underlining the importance of detection of HAV viraemia. However, data on the duration of HAV excretion remain limited.

Because acute HAV infection is self-limiting, longitudinal studies in which clinical data are collected along with samples are uncommon. For a follow-up study, blood and faecal samples were collected from 27 patients with acute hepatitis A infection for a maximum period of 26 weeks. Our aim was to determine the median duration of HAV excretion in faeces and blood and thus the period of possible infectiousness. Additionally, in 55 other patients whose HAV had been typed [van Steenbergen et al., 2004], it was investigated whether the degree of liver damage affected duration and level of HAV excretion in faeces and viraemia level, and whether HAV genotype affected the duration of HAV excretion and jaundice.

## MATERIALS AND METHODS

### Participants

For the follow-up study, all patients who were positive for anti-HAV immunoglobulin M (anti-HAV IgM(+)) and reported between August 2003 and August 2004 to the Public Health Services in Amsterdam, Brabant, South Limburg, Groningen, South-Holland South and South-Holland North were approached.

Participants were asked to sign an informed consent form and to donate a maximum of nine faecal samples and three blood samples over 26 weeks. To prevent distress in children younger than 12 years, they were not required to donate multiple blood samples, but only the one sample used for detecting anti-HAV IgM. The participants were divided at random into groups A and B to donate on different schedules (Table I) to obtain a maximum number of time points after infection. In weeks 1–4 after start of symptoms, participants donated three faecal samples; in weeks 5–26 they donated one faecal sample every 4 weeks. If three consecutive faecal samples were negative in the HAV PCR before the end of the 26-week period, follow-up for that patient was stopped. Adults were asked to donate three blood samples in the first 4 weeks of illness, but a few samples were received after more than 4 weeks. Participants were asked to complete a structured questionnaire each time they sent in a faecal sample. It asked about the presence and duration of symptoms such as jaundice, vomiting, nausea, fatigue, medications and co-infections. The blood samples were used to determine levels of ALT to quantify liver damage. Patients with ALT levels above normal, that is, >45 IU/L blood (37°C), were considered to have abnormal liver function.

Additionally, blood samples were used representing 55 sporadic HAV cases who participated previously in an HAV typing study [Bruisten et al., 2001; van

TABLE I. Scheme for Sample Donation

| Weeks after onset of illness | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group A | F B | | F B | | F B | | F | | | | F | | | | F | | | | F | | | | F | | | | F |
| Group B | F B | F B | | F B | | F | | | F | | | | F | | | | F | | | | F | | | | F | | |

Patients donated samples according to group A or B.
In weeks 1–4 after onset of illness, they gave three blood and three faeces samples. In weeks 5–26, they donated one faeces sample every 4 weeks. If three consecutive faeces samples were negative in the HAV RT-PCR, the patient was informed that he had completed the study.
F, Faeces sample; B, Blood sample.

Steenbergen et al., 2004], to study the relation between the degree of liver damage and the virus load. Patients were reported from December 2000 to February 2004 to the Amsterdam health service (HS).

### Time Definition

Because the production date of the faecal samples was often unknown, the date on which it was received was used. The sampling time was calculated as the days between the first day of symptoms (jaundice) and the date the sample was received. Sampling time in blood was the number of days between the first day of symptoms and the donation date.

Blood and faecal samples from individual follow-up participants were considered to be measured in the same period (i.e. one measurement), if faeces were provided within 5 days after giving a blood sample.

### RNA Isolation, Detection, Sequencing and Phylogenetic Analysis

RNA extraction, nested RT-PCR, sequencing and phylogenetic analysis were carried out as described previously [Bruisten et al., 2001; van Steenbergen et al., 2004; Tjon et al., 2005]. The detection RT-PCR was carried out in an end volume of 25 µl with an input of 5 µl RNA from patient material.

Random hexamer primers were used for reverse transcription. PCR primers for nested amplification of the VP3-VP1 region and the VP1-P2a region of the HAV genome were used as described previously [Bruisten et al., 2001; van Steenbergen et al., 2004; Tjon et al., 2005]. All primers were synthesised by Isogen Life Science (Maarssen, The Netherlands).

### Construction of Competitor RNA

The competitor was created from RNA isolated from virus culture $HAV_{cyt/HB1.1}$ [Brack et al., 1998] with Tripure isolation reagent (Roche, Almere, The Netherlands). Full-length HAV RNA was resuspended in 50 µl Tris-HCl (10 mM, pH 8.0) and stored at −80°C until use.

The method was adapted from Diviacco et al. [1992]. Two RT-PCRs were performed with the Access RT-PCR kit (Promega, Leiden, The Netherlands). The sequences of the primers used in the RT-PCR reactions are shown in Table II. The 25 µl RT-PCR mixtures contained 0.5 µl $HAV_{cyt/HB1.1}$ RNA, 1× AMV/Tfl reaction buffer, 200 µM dNTP, 1.5 mM $MgSO_4$, 10 ng of the sense and the anti-sense primer, 0.1 U AMV reverse transcriptase and 0.1 U Tfl DNA polymerase. The RT step was carried out at 48°C for 45 min and 94°C for 2 min. Subsequently, 35 amplification cycles were carried out at 94°C for 30 sec, 55°C for 30 sec, 68°C for 90 sec, and terminated with 68°C for 7 min. The amplification products of RT-PCR1 (175 bp) and RT-PCR2 (222 bp) contain an overlapping region of 20 bp with restriction sites EcoRI and HindIII (Table II). This region was used for 1:1 hybridisation of PCR1 and PCR2 amplimers. Subsequently, 0.2 µl of the mixture and primers VP1-4-sense-NcoI and VP1-6-SalI (Table II) were added in a 25 µl PCR mix and amplification was carried out [Bruisten et al., 2001]. The resulting 397 bp amplimer was verified on a 1% agarose gel, isolated with the Qiagen gel extraction kit (Qiagen Benelux B.V., Venlo, The Netherlands) and dissolved in 50 µl Tris-HCl (10 mM, pH 8.0). The fragment was cloned into a pGEM5Zf(+) vector and grown in JM109 bacteria (Promega Benelux B.V., Leiden, The Netherlands). Plasmid DNA was isolated from a recombinant clone and linearised by incubation at 95°C for 2 min and placement on ice for 5 min. Competitor RNA was made using T7 polymerase (Promega Benelux B.V.) and contaminating DNA was removed with RQ1 Dnase (Promega Benelux B.V.) according to the manufacturers' protocols.

### Quantitative RT-PCR

Serial dilutions of competitor RNA were amplified by nested PCR with and without an RT step according to Bruisten et al. [2001] to determine the presence of contaminating DNA. Dilution samples that were negative in the PCR reactions without the RT step were used for quantitative RT-PCR with patient material. Two methods were used for calculating the concentration of the competitor RNA. The first method was based on the OD260 value measured by spectrophotometer of the competitor RNA, which was 0.024, and the Avogadro formula. The total length of the competitor RNA is 443 nucleotides, resulting in a concentration of the competitor RNA starting solution of $4 \times 10^{11}$ copies/µl. The second method was based on the fact that RNA detection by RT-PCR is possible from 10 to 20 copies per reaction, assuming a 10% efficiency of the cDNA step and the potency to detect 1–2 copies of target cDNA. Using this method we calculated that the concentration of the competitor RNA starting solution was about $4 \times 10^{12}$ copies/µl. The average of these numbers was calculated

TABLE II. Primer Sequences Used in the Two RT-PCRs to Construct the Competitor

| | Primer | Sequence 5′-3′ | Nucl nr[a] |
|---|---|---|---|
| RT-PCR1 | VP1-4s NcoI | TAC/CATGGcgttgcttcccatgtcaga | 2131–2149 |
| | VP-mut-as EcoRI/HindIII | ccGAATT/CcgcgAAGC/Ttgcaacattctgttctgtagaaa | 2259–2278 |
| RT-PCR2 | VP1-6 SalI | GCG/TCGACcatatgatctgatgtatgt | 2455–2473 |
| | VP-s-mut EcoRI/HindIII | gcA/AGCTTcgcgG/AATTCggccagatccccaagttgtat | 2279–2298 |

[a]Nucleotide numbering is according to strain $HAV_{cyt\ HB1.1}$ [Brack et al., 1998]. The underlined part of the primer sequences written in capital letters indicates restriction sites. The EcoRI/HindIII sites are in a 20 bp region, which is present in the competitor sequence but not in wild-type HAV.

and the competitor concentration was set at $2 \times 10^{12}$ copies/μl.

Serial tenfold dilutions of the competitor RNA were divided into aliquots with Tris-HCl (10 mM, pH 8.0) containing 0.5 U/μl Rnasin. The quantitative RT-PCR was performed according to the HAV RT-PCR protocols described previously [van Steenbergen et al., 2004; Tjon et al., 2005] in an end volume of 25 μl with an input of 2 μl competitor RNA and 2 μl RNA from patient material. Amplification of the competitor resulted in an amplicon of 267 bp, while the amplicon size of patient-derived HAV cDNA was 247 bp. The qualitative RT-PCR had a higher sensitivity than the quantitative RT-PCR concerning detection of patient-derived HAV RNA because no competitor RNA was added. Hence, HAV RNA detected with the qualitative RT-PCR was not always quantifiable. The number of HAV copies in a stool sample were calculated per millilitre of a 10%–20% suspension of fresh stool in phosphate buffered saline; hereafter referred to as "faeces suspension". Quantitation was possible from virus load levels of $2 \times 10^3$ copies/ml faecal suspension and from $1 \times 10^3$ copies/ml blood.

### Statistical Analysis

Statistical analyses were performed with SAS [SAS statistical package, 2005b], R [R statistical package, 2005a] and SPSS 12.0 [SPSS 12.0, 2005c] software for Windows. The distribution of the time until virus loads in faeces and blood were below a certain level was estimated, for example undetectable by RT-PCR, using the nonparametric maximum likelihood estimator for interval censored data [Groeneboom, 1996].

Relations between the three markers (ALT and virus levels in blood and in faeces) were analysed in a linear regression model after transformation to the log-scale. For each marker combination, two models were fitted; each marker was used as dependent variable to predict the other.

The Mann–Whitney test was used to determine if HAV genotype influenced duration of HAV excretion in faeces and/or jaundice. The test was also used to determine if liver damage influenced the duration of HAV excretion, comparing patients with ALT levels above and below the median of 1,000 IU/ml blood.

## RESULTS

### General Characteristics of Participants

***Follow-up study.*** Of the anti-HAV IgM positive cases reported from August 2003 to 2004 at the participating health services, 27 people participated in the follow-up study. As shown in Table III, these included 18 men and 9 women (median age 33 years; interquartile range (IQR) 23–42 years), of whom 17 persons were infected with subtype 1a strains (63%), 7 with subtype 1b strains (26%) and 3 with subtype 3a strains (11%).

All but two participants reported jaundice. Of these, 84% (21/25) also reported the duration of jaundice, which lasted for a median period of 17 days (IQR: 5–74 days). Comparing patients infected with genotype 1a versus genotype 1b, we found no significant difference in duration of jaundice (Mann–Whitney test: $P = 0.89$) or duration of HAV excretion in faeces ($P = 0.13$).

TABLE III. Characteristics of the Participants in the Follow-Up and the HAV Typing Study

| | Follow-up study | HAV typing study |
|---|---|---|
| No. of patients | 27 | 55 |
| Median age (years) | 33 (IQR 23-42) | 32 (IQR 12-38) |
| Gender | | |
| Male | 18 (67%) | 43 (78%) |
| Female | 9 (33%) | 12 (22%) |
| Genotype | | |
| 1a | 17 (63%) | 39 (71%) |
| 1b | 7 (26%) | 14 (25.5%) |
| 3a | 3 (11%) | 2 (3.6%) |
| Jaundice | 25/27 (93%) | 49/55 (89%) |
| Median duration of jaundice (days) | 17 | NA |
| Median duration of HAV excretion (days) | 81 | NA |

NA, not applicable.

### HAV-Typing Study Participants

These 55 patients consisted of 43 men and 12 women (median age: 32 years; IQR 12–38 years) (Table III). There were 39 persons infected with subtype 1a strains (71%), 14 with subtype 1b (25.5%) and 2 with subtype 3a (3.6%). Of the total, 49 persons (89%) reported jaundice.

### Time Course of Virus Load Levels and ALT Among Patients

In concordance with the comparisons mentioned above, there were no observable differences per genotype concerning the course of HAV excretion. In The Netherlands, there are two main risk groups among whom different HAV genotypes circulate: homosexual men are infected with genotype 1a strains, while travelers to HAV endemic countries are infected mainly with either genotype 1b strains (Morocco, Turkey, etc.) or genotype 3a strains (Pakistan, India) [van Steenbergen et al., 2004]. The patterns of patients infected with genotype 1a, 1b or 3a are shown in Figure 1a–c.

Figure 1a represents a 26-year-old homosexual man infected with a genotype 1a strain. He reported anonymous sex in saunas as a risk factor. He was jaundiced for 16 days and a HAV faecal excretion was detected up to 98 days after disease onset.

Figure 1b depicts a 31-year-old patient who was infected in Morocco with a genotype 1b strain. He was jaundiced for 21 days, and virus excretion was detected until 70 days after onset of illness.

Figure 1c represents an 11-year-old patient who became infected in Pakistan with a genotype 3a strain. The patient was jaundiced for 18 days and HAV excretion was detected for 76 days.

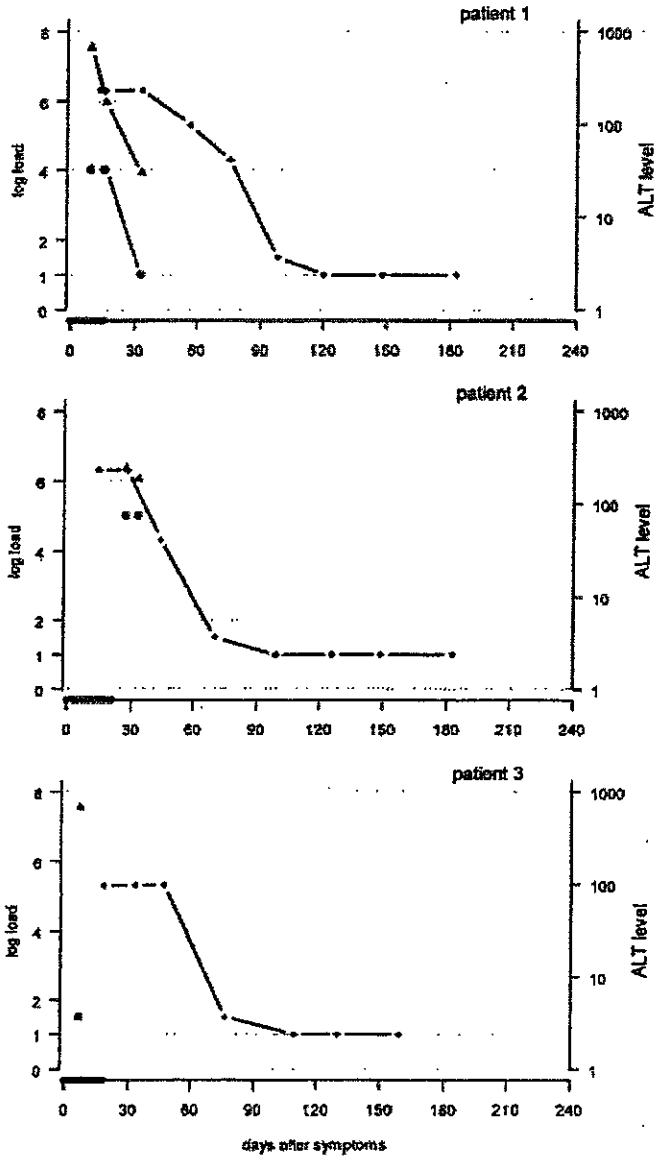


Fig. 1. a–c: Time course of virus load and ALT level in three patients. The left Y-axis shows the logarithm of virus load in linear scale per ml faeces suspension or per ml blood. Logload = 1: detection limit qualitative RT-PCR. Logload = 1.5: detection limit quantitative RT-PCR. Logload = 2: Quantification limit. The right Y-axis shows the ALT levels in log scale in IU/ml blood. The X-axis shows the number of days after onset of symptoms. Duration of jaundice is depicted by the grey bar on the X-axis. a: Patient 1 is a 26-year-old homosexual man who got infected with a type 1a virus by anonymous sex in public venues. He was jaundiced for 16 days. b: Patient 2 is a 31-year-old man who got infected with a genotype 1b strain in Morocco. He was jaundiced for 21 days. c: Patient 3 is an 11-year-old boy who got infected with a genotype 3a strain in Pakistan. He was jaundiced for 18 days.

## HAV Detection and Quantitation in Faeces

Hepatitis A virus (HAV) faecal excretion was detected for an estimated median period of 81 days after onset of symptoms in samples of the 27 follow-up study participants (Fig. 2). Faecal excretion was never shorter than 57 days, with a maximum of 127 days. Fifty percent of the participants still excreted at least $2 \times 10^6$–$2 \times 10^8$ copies/ml faeces suspension at 36 days after onset of

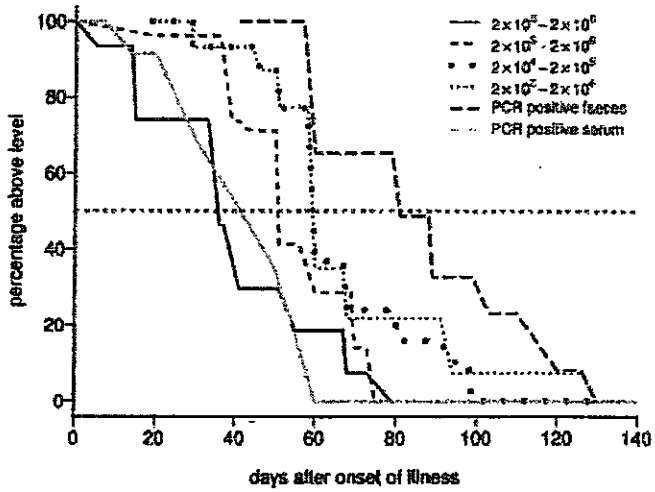


Fig. 2. Curves for virus excretion levels at different time points. The black curves show the estimated percentage of patients over time that have a virus load in faeces above a certain limit. The grey curve shows the last time points at which sera were HAV-positive in the qualitative RT-PCR. The horizontal line is the median value.

symptoms. After 65 days this had declined to 10% of patients. The lowest quantifiable level, $2 \times 10^3$ copies/ml faeces suspension, was detected for a median period of 60 days.

## HAV Detection and Quantitation in Blood

In the follow-up study (n = 27), serum samples were provided from 1 day before onset of illness to 74 days after the onset of symptoms. The estimated median period in which HAV was detected in blood was 42 days after disease onset (Fig. 2). At 60 days after the onset, all blood samples were estimated to be HAV-negative.

The PCR results of the sera provided by participants of both the follow-up and HAV typing study are shown in Figure 3. In total, 119 samples taken from 82 participants were tested, since 22 follow-up patients donated more than one blood sample. Again, we found that the proportion of PCR-positive sera declined over time. It

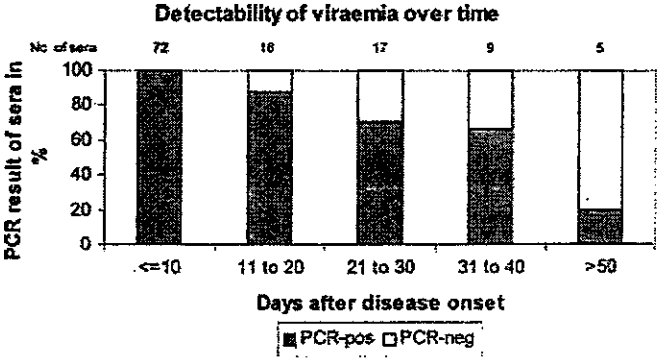


Fig. 3. Detectability of HAV viraemia according to days after onset of illness. The Y-axis shows the percentage of PCR-positive (grey) blood samples donated by participants of the follow-up and the HAV-typing study. All 72 sera (100%) donated within the first 10 days of illness were PCR-positive, whereas 80% of the sera donated more than 40 days after onset were PCR-negative.

was 100% in the period up to 10 days after onset of illness and declined to 20% after 40 days (Fig. 3).

Taking into account one serum sample per patient obtained at different time points after the onset of disease, it was found that the median virus load levels varied at different time points. In the first 10 days of illness, the median virus load level was $1 \times 10^6$ copies/ml blood (95% confidence interval (CI): $5.2 \times 10^5$–$1.9 \times 10^6$ copies/ml blood; n = 69). After 10 days, the median virus load of the donated sera was below quantitation level ($<10^3$ copies/ml).

### Relation Between ALT Levels and Virus Levels in Blood and in Faeces

***ALT levels and virus load in blood.*** To determine if the degree of liver damage influenced virus levels in blood in the first 10 days of illness and vice versa, data were analysed from follow-up and HAV typing-study participants (n = 64). Spearman analysis revealed a significant association between liver damage and the HAV level in serum ($\rho = 0.354$; $P = 0.004$). This was corroborated by the results from linear regression analysis, which showed that if ALT levels decreased with $1\log_{10}$ IU/L blood, viraemia levels decreased with $0.81\log_{10}$ copies/ml blood ($P = 0.017$), and vice versa, if viraemia levels decreased with $1\log_{10}$ copies/ml blood, ALT levels decreased with $0.11\log_{10}$ IU/L blood ($P = 0.017$). To minimize the confounding effect of time, these analyses were repeated with data of the first 5 days of illness, which yielded similar results.

***ALT levels and virus load in faeces.*** Nine participants provided blood and faeces in the same period (5 days) within the first 10 days of illness. Using Spearman analysis, a strong positive correlation was found between the virus level in blood and in faeces ($\rho = 0.942$; $P < 0.01$). No significant correlation was found between ALT levels and virus load in faeces during this period ($\rho = 0.248$; $P = 0.520$).

***ALT levels and duration of HAV excretion in faeces.*** The possible relation between liver damage and the duration of HAV excretion in faeces was of interest. Upon analysis, no difference was found in duration of HAV excretion between persons with ALT levels above the median (1,000 IU/ml blood) versus ALT levels below the median in the first 10 days of illness (Mann–Whitney test: $P = 0.5$; n = 16).

## DISCUSSION

Due to the acute and self-limiting nature of HAV infection, sequential collection of samples combined with clinical data from patients is uncommon. Information on the duration and level of HAV excretion in blood and in faeces is therefore limited despite its importance for disease control.

To elucidate these matters, clinical data and serial blood and faeces samples were collected from 27 acute hepatitis A patients for half a year. The biochemical and clinical data were combined with quantitative RT-PCR results to study possible relations between symptomatic disease and the duration and level of HAV excretion.

In other studies, the real-time PCR method was used to quantify HAV loads in sera. These real-time PCRs target the 5′ noncoding region and virus loads were quantified with an external standard [Costa-Mattioli et al., 2002b; Rezende et al., 2003; Normann et al., 2004].

At the onset of this study, a real-time PCR was compared with a competitive nested RT-PCR, targeting the VP3-VP1 region (data not shown). An internal standard was used for both virus quantitation, and as an inhibition control for PCR of faeces-derived RNA samples. Tests using the real-time PCR showed a significant increase in the fluorescent signal representing amplification of the internal standard, whereas almost no increase in fluorescent signal was detected for amplification of the wild-type HAV cDNA. Nevertheless, both the internal standard and the wild-type HAV cDNA were amplified during the reaction, since amplicons of the expected sizes were visible on acryl-amide gels. Unfortunately, we were not able to solve this problem and therefore chose to use the competitive nested RT-PCR that is described in the present study, as this yielded more reproducible and robust results.

The results show that HAV is detectable in faeces for a median period of 81 days after onset of symptoms, with a maximum of 127 days. Although the literature on this subject is sparse, studies with fewer patients describe similar findings of HAV excretion periods in faeces from 52 days post-onset up to 89 days in healthy individuals [Yotsuyanagi, 1996; Polish et al., 1999; Flehmig et al., 2000; Chitambar et al., 2001; Costa-Mattioli et al., 2002a]. HIV-positive persons may excrete the virus for even longer periods, since one study found that an HIV-1 infected person still excreted $5 \times 10^6$ copies/g faeces 106 days after the onset of illness [Costa-Mattioli et al., 2002a].

Of our patients, 50% were still shedding HAV in faeces at 36 days, at the same high levels as at disease onset (Fig. 2). One study reported successful infection of two tamarins by inoculation with a suspension of faeces donated 27 days after the ALT peak of the human patient [Polish et al., 1999]. The animals seroconverted and excreted HAV in blood and in faeces after 15 and 21 days, respectively. Thus, patients still excreting high levels of HAV in faeces after 1 month may still be infectious.

Sera collected during the present study were HAV RNA-positive for a median period of 42 days after illness. Other studies report viraemic periods varying from 22 up to 490 days [Bower et al., 2000; Kwon et al., 2000; Ida et al., 2002; Costa-Mattioli et al., 2002b; Sagnelli et al., 2003; Normann et al., 2004].

Although HAV transmission by blood products is rare, there have been several reports on HAV outbreaks due to contaminated blood products [Mannucci et al., 1994; Robertson et al., 1998; Soucie et al., 1998; Chudy et al., 1999; Gowland et al., 2004; Heitmann et al., 2005]. To prevent contamination of blood products in The Netherlands, HAV patients are excluded from donating

blood for 4 weeks after clinical symptoms are resolved, or 6 weeks in the case of contact with a HAV patient. Since the patients had jaundice for a median period of 17 days, and elevated ALT levels for a median period of 16 days, the present study suggests that most HAV patients are probably HAV RNA-negative by the end of the 4–6 weeks of exclusion. However, blood donors without any HAV symptoms and those with prolonged viraemia must also be taken into account, especially since donated blood is not tested for the presence of HAV in The Netherlands. An even greater risk to blood supplies is present during the period before HAV antibodies, increased liver enzyme levels and HAV symptoms are detected [Gowland et al., 2004; Heitmann et al., 2005]. Weimer et al. [2002] reported a window period of 2 weeks in which HAV-infected patients had high HAV levels in blood with normal ALT levels before onset of illness. PCR techniques can reduce this window period and increase the safety of blood products, especially since HAV is 10,000 times more infectious by the intravenous route than orally, and contaminated blood products can increase morbidity and mortality in already ill patients treated with these products [Purcell et al., 2002].

It was found that in the first 10 days after onset of illness, ALT levels were correlated positively with viraemic levels in blood. However, no indications were found that the degree of liver damage during this period influenced duration of HAV excretion in faeces. Other studies have found that ALT and HAV levels in blood are correlated positively [Fujiwara et al., 1997; Normann et al., 2004; Sainokami et al., 2005]. The results of the present study support the findings of Bower et al. [2000] who detected HAV levels of $10^4$–$10^5$ plaque forming units (pfu)/ml up to 20 days prior to the ALT peak. These levels declined to 1–10 pfu/ml 30 days after the ALT peak.

The factors that cause liver damage during HAV infection are still poorly understood. One hypothesis is that hepatic disease is caused by the patient's immune system as it attempts to clear the virus [Vallbracht et al., 1986; Brack et al., 2002; Rezende et al., 2003]. Age and underlying disease, as well as medication, can influence disease outcome. Rezende et al. [2003] reported that patients with HAV-related fulminant hepatitis had low HAV levels in blood in spite of hepatocyte necrosis. This finding is in agreement with Sainokami et al. [2005] who found that ALT levels and virus load were significantly correlated in patients with mild HAV-related symptoms, but not in those with severe symptoms. In contrast to the position in vivo, wild-type HAV can persistently infect human cell lines without causing observable cytopathogenicity [Vallbracht et al., 1984]. Cytotoxic peripheral blood lymphocytes taken from HAV patients have been shown to induce cytolysis in HAV-infected cell cultures, whereas lymphocytes from noninfected persons did not have this effect [Vallbracht et al., 1986; Maier et al., 1988]. Accordingly, one would expect immunocompromised persons to have persistent HAV infection. Reportedly, immunocompromised HIV-infected persons are HAV-viraemic for a longer period than expected [Ida et al., 2002; Costa-Mattioli et al., 2002a] and one study found that they show less elevated ALT levels and are longer viraemic than HIV-negative patients [Ida et al., 2002]. Another study found that neonates still excreted HAV 4–5 months after infection [Rosenblum et al., 1991]. Hence, our finding that the virus load in blood is associated positively with liver damage supports the notion that HAV clearance in hepatocytes is immunomodulated.

On the other hand, increased disease severity may be associated with viral factors, such as genotype and nucleotide changes in, for example the 5′-non-translated-region of the virus [Fujiwara et al., 2002]. No indication was found in this study that genotype influenced duration of jaundice or HAV excretion, nor did other studies looking for an association between HAV genotype and disease severity [Fujiwara et al., 2003; Rezende et al., 2003; Normann et al., 2004]. The findings show that HAV faecal excretion is high in the first month of illness, suggesting that patients may be infectious for a longer period than currently assumed. Furthermore, HAV patients can be viraemic for up to 2 months, which has to be taken into account by blood banks. Finally, a positive association was found between ALT and viraemia levels during the first 10 days of illness.

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**研究報告の概要**

○分子疫学的試験によりヨーロッパにおける活動的男性同性愛者のA型肝炎エンデミックが示されている
1997年～2005年に、デンマーク、ドイツ、オランダ、ノルウェー、スペイン、スウェーデン、英国において、男性同性愛者にA型肝炎の大規模アウトブレイクが発生した。このA型肝炎アウトブレイクに関与するウイルス株に遺伝子学的な関連性があるかどうかを確かめるため、これら国々の間で共同試験が実施された。当該株のVP1およびVP1/P2A領域のN末端の配列を解析し比較した。ヨーロッパのさまざまな国の男性同性愛者に認められた株のほとんどは、MSM1と名づけられたgenotype IA に属する近縁のクラスターを形成していた。同じ時期に、これらの国々の別のリスク集団には異なるHAV株が流行しており、このことは男性同性愛者だけに特定のHAV株が流行したことを示している。1997年～2005年の期間に男性同性愛者に同一の株が認められたことは、これらHAV株が男性同性愛者間に長く流行していることを示している。各国内の同性愛者コミュニティーはその集団内にHAVを長期間維持するにはおそらく小さすぎる。しかし、ヨーロッパ全土の同性愛者コミュニティーは相同性の高いHAV 株の流行が何年も持続するのに十分な大きさであるため、男性同性愛者間での流行が引き起こされたのであろう。

**使用上の注意記載状況・その他参考事項等**

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 1997年～2005年に、ヨーロッパにおいて男性同性愛者間で特定のHAV株によるA型肝炎の大規模アウトブレイクが発生したとの報告である。 | 日本赤十字社は、輸血感染症対策として、問診で肝炎の既往があった場合、A型肝炎については治癒後6ヶ月間、家族に発症した人がいる場合は1ヶ月間献血不可としている。また、男性と性的接触を持った男性は1年間献血不可としている。今後も引き続き情報の収集に努める。 |

(17)

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# Molecular Epidemiological Studies Show That Hepatitis A Virus Is Endemic Among Active Homosexual Men in Europe

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Large outbreaks of hepatitis A have occurred in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom during the period 1997–2005 affecting homosexual men. A collaborative study was undertaken between these countries to determine if the strains involved in these hepatitis A outbreaks were related genetically. The N-terminal region of VP1 and the VP1/P2A region of the strains were sequenced and compared. The majority of the strains found among homosexual men from the different European countries formed a closely related cluster, named MSM1, belonging to genotype IA. Different HAV strains circulated among other risk groups in these countries during the same period, indicating that specific strains were circulating among homosexual men exclusively. Similar strains found among homosexual men from 1997 to 2005 indicate that these HAV strains have been circulating among homosexual men for a long time. The homosexual communities are probably too small within the individual countries to maintain HAV in their population over time, whereas the homosexual communities across Europe are probably sufficiently large to sustain continued circulation of homologous HAV strains for years resulting in an endemic situation among homosexual men.
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**KEY WORDS:** molecular epidemiology; HAV; outbreaks; homosexual men

## INTRODUCTION

Hepatitis A is transmitted primarily by the fecal-oral route from person to person. The seroprevalence in Europe varies from intermediate levels in Southern and Eastern Europe to low levels in Western Europe, and very low levels in Scandinavia [Jacobsen and Koopman, 2004]. In low endemicity countries, hepatitis A mainly affects groups at high risk of infection such as homosexual men, intravenous drug users, and travellers to high endemicity areas. In recent years, several outbreaks occurred among homosexual men in several countries in Europe: United Kingdom in 1997 [Bell et al., 2001], Norway in 1997–98 [Stene-Johansen et al., 2002], Germany in 1997 [Fengler, 1997], and 2002–2003 (unpublished), France in 2000 [Delarocque-Astagneau, 2004], and Spain in 2002 and 2003–2004 (unpublished), and in addition, the Netherlands reported sporadic cases in 1997–1998 [Bruisten et al., 2001] and 2000–2004 [De Jager and Heijne, 2004; Reintjes et al., 1999; van Steenbergen et al., 2004]. In 2004, outbreaks of hepatitis A among homosexual men were reported in Denmark [Mazick et al., 2005], Norway [Blystad et al., 2004], Sweden (unpublished), the United Kingdom [O'Sullivan, 2004], and Spain (unpublished).

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This suggests that HAV is transmitted readily within the homosexual communities on a national basis and perhaps on an international basis as well. Whether HAV was transmitted between homosexual communities in the different European countries, is not known. The objective of this study was to determine if these viral outbreaks among homosexual men were related genetically by comparing sequences of HAV outbreak strains from the individual countries, as well as studying the dissemination of these strains in the population. If the HAV strains found among homosexual men are not found in the general population or other risk groups within the individual countries during the same time, this could indicate that these strains circulate among homosexual men exclusively. Phylogenetic analysis of the strains found among homosexual men and other HAV strains is presented, and discussed in relation to endemic HAV transmission among homosexual men.

## MATERIALS AND METHODS

An epidemiological and molecular collaborative study of hepatitis A among homosexual men was established between surveillance centres and laboratories in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. Epidemiological data regarding place of infection, age, onset of illness, and sexual orientation was obtained during the time period of outbreaks associated with homosexual men in the individual countries in 1997–2005 by the national surveillance centres. Cases suspected to be associated with the outbreaks among homosexual men were characterized further by sequencing of HAV strains. Based upon the molecular epidemiological data, cases were defined as men ≥17 years of age with acute hepatitis A infection, verified by positive anti-HAV IgM and classified by three case definitions to identify homosexual-related transmissions;

- 1. Confirmed case: HAV outbreak strain confirmed by sequencing and notified as sexually transmitted.
- 2. Probable case: HAV outbreak strain confirmed by sequencing, they had not been notified as sexually transmitted or other known risk factors such as drug use or travel to high endemic regions.
- 3. Possible case: HAV outbreak strain not detected and no other known risk factors had been notified such as drug use or travel to high endemic regions.

Women and children <17 years of age were excluded from the study.

### Isolation, Amplification, and Sequencing of HAV RNA

HAV RNA was isolated, amplified, and sequenced by the laboratories in the respective countries according to the protocols detailed below. Table I shows the different extraction and sequencing methods, as well as primers used by each country. The PCR products were subsequently sequenced with second PCR primers.

#### Denmark

One-step RT-PCR (QIAGEN, GmbH, Hilden, Germany) was used to amplify the N-terminal part of VP1 and the VP1/P2A junction of HAV as previously described [Grinde et al., 1997; Arauz-Ruiz et al., 2001].

#### Germany

cDNA was synthesised from 5 µl RNA with 1.25 µM HAV5 primer using M-MLV Reverse Transcriptase (Invitrogen, Carlsbad, CA). The VP1/P2A and VP3/VP1 regions were amplified by nested PCR using 0.5 µM primer, 1.5 mM $MgCl_2$, 0.5 U Taq-polymerase and amoniumsulfate buffer (Invitek GmbH, Berlin, Germany). The amplifications were performed for 35 cycles at 94°C in 30 sec, 42°C in 1 min, 72°C in 2 min.

#### The Netherlands

Isolation, amplification, and sequence analysis of the HAV strains found among Dutch patients were performed as described previously [Bruisten et al., 2001; van Steenbergen et al., 2004; Tjon et al., 2005a].

#### Norway

cDNA was synthesized and subsequent PCR amplifications were generated with AmpliTaq DNA polymerase (Applied Biosystem, Branchburg, NJ) with 0.5 µM primers, 2 mM $MgCl_2$, and 200 µM dNTP as previously described [Grinde et al., 1997]. The first and second PCR amplifications were performed for 30 and 35 cycles respectively at 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec. In the second PCR (VP1/P2A), the annealing temperature was 60°C.

#### Spain

Two overlapping fragments were amplified by using two sets of primers: the $NH_2$-VP1 pair and the COOH-VP1 pair [Sanchez et al., 2002, 2003a,b]. Ten microliters of the extracted nucleic acids were used for the cDNA synthesis in two separate RT reactions using the primers NH2-VP1 or COOH-VP1. Ten microliters of the RT products were added to 50 µl final volumes of PCR mixes. The amino-VP1 PCR was amplified with 53°C annealing temperature using 2 mM $MgCl_2$, while the carboxy-VP1 was amplified with 45°C annealing temperature using 2.5 mM $MgCl_2$. The Expand enzymes from Roche Diagnostics (Basel, Switzerland) were used.

#### Sweden

cDNA synthesis as described previously [Arauz-Ruiz et al., 2001]. The N-terminal of VP1 and the VP1/P2A region were amplified by PCR as described previously [Grinde et al., 1997; Tallo et al., 2003]. The nested PCR

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TABLE I. Extraction and Sequencing Methods, and Primers Used by Each Country

| Country | Extraction and sequencing method | Region | Primer (sequence or reference) |
|---|---|---|---|
| Denmark | -MagNa-Pure sample extraction | VP1/P2A | HAV6, HAV7, HAV8, HAV9 [Grinde et al., 1997] |
| | -ABI 3130XL genetic analyzer using BigDye Terminator chemistry (Applied Biosystem) | VP3/VP1 | HA1, HA7, 2167, HA3 [Arauz-Ruiz et al., 2001] |
| Germany | -QIAamp viral RNA Kit (Qiagen) | VP1/P2A | HAV5 3373 5′- CCATTTCAAGAGTCCACACAC-3′* |
| | -ABI Prism 377 DNA sequencer with Big Dye Terminator chemistry (Applied Biosystem) | | HAV6 2791 5′- ATTCAGATTAGACTGCCTTGGTA-3′* |
| | | | HAV7 3276 5′- CATTATTTCATGCTCCTCAGT-3′* |
| | | | HAV8 2906 5′- GGTTTCTATTCAGATTGCAAATTA-3′* |
| | | VP3/VP1 | HAV1 2443 5′- GATCTGATGTATGTCTRGAYTC-3′* |
| | | | HAV2 2125 5′- GTTAATGTTTATCTTTCAGCWAT-3* |
| | | | HAV3 2407 5′- CAGGAAATGTCTCAGGYACTTTCT-3* |
| | | | HAV4 2164 5′- GCTCCTCTTTATCATGCTATGGAT-3* |
| The Netherlands | -TriPure Isolation Reagent (Roche) | VP1/P2A | BR-5, BR-9, RJ-3, BR-7 [Bruisten et al., 2001; van Steenbergen et al., 2004] |
| | -ABI Prism 310 genetic analyzer with Big Dye Terminator chemistry (Applied Biosystem) | VP3/VP1 | VP1-4 sense, VP-6 antisense, VP1-2 sense, VP1-1 antisense [Bruisten et al., 201; van Steenbergen et al., 2004] |
| Norway | -QIAamp viral RNA Kit (Qiagen) | VP1/P2A | HAV6, HAV7, HAV8[Grinde et al., 1997] |
| | -ABI Prism 310 genetic analyzer with Big Dye Terminator chemistry (Applied Biosystem) | VP1 | VP1-4 sense [van Steenbergen et al., 2004] |
| | | | VP1-anti-sense 2659 3′- AGGCCATGCCATCTACATCAG-5′[?] |
| | | | VP1-nested-sense 2170 5′- TTGCTCCTCTTTATCATGCTATG-3′[?] |
| Spain | -Guanidine thiocyanate [Boom et al., 1990] | VP3/VP1/P2A | $NH_2$-VP1 primer pair, COOH-VP1 primer pair [Sanchez et al., 2003b] |
| | -ABI Prism 377 DNA sequencer with Big Dye Terminator chemistry (Applied Biosystem) | | |
| Sweden | -TRIZOL® LS reagent (Invitrogen) | VP1 | HA2112S, HA1865AS, HA1369S [Tallo et al., 2003] |
| | -ABI Prism 3100 genetic analyzer with Big Dye Terminator chemistry (Applied Biosystem) | VP1/P2A | HAV6, HAV7, HAV8, HAV9[Grinde et al., 1997] |
| United Kingdom | -QIAamp Ultrasens Virus Kit (Qiagen) | VP1/P2A | HAV6, HAV7 [Grinde et al., 1997] |
| | -CEQ8000 sequencer with GenomeLab™ DTCS Quick Start Kit (Beckman Coulter, Fullerton, CA) | | BR-9, BR-5 [Robertson et al., 1992] |

*The primers positions are given according to strain HPACG (M20273).
[?]The positions of the primers are according to reference strain HM-175 (M14707).

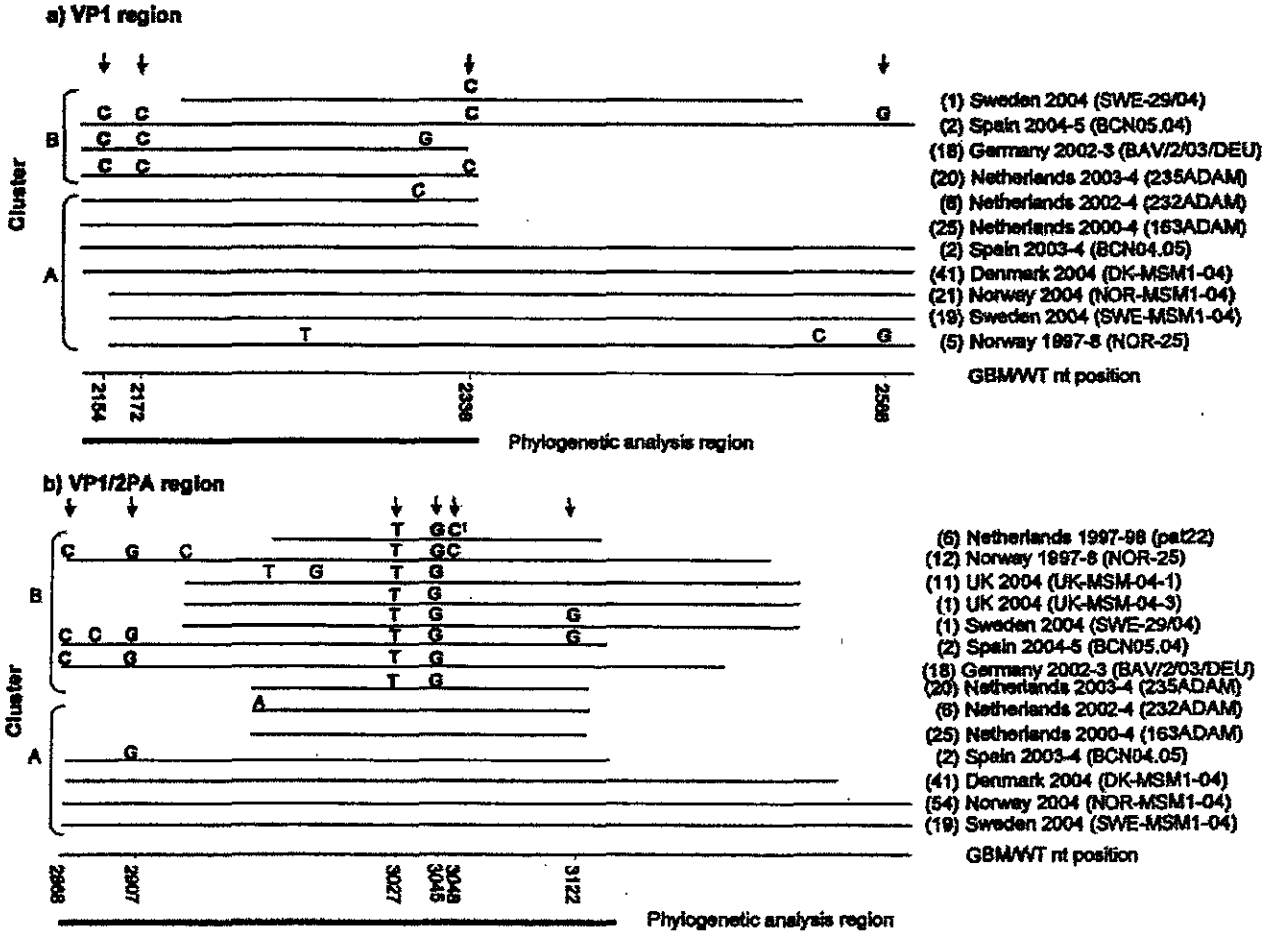
products were purified and sequenced as described previously [Tallo et al., 2003].

### The United Kingdom

HAV RNA was reverse transcribed into cDNA using random hexamers (GE Healthcare, Buckinghamshire, UK) and M-MLV reverse transcriptase (Invitrogen). Primary amplification of the VP1/P2A junction was amplified as previously described [Grinde et al., 1997]. The secondary amplification was performed with primers BR-9 and BR-5 [Robertson et al., 1992] and MegaMix Blue PCR master mix (Microzone Ltd., Ottawa, Canada) using the cycling conditions previously described [Bruisten et al., 2001].

### Phylogenetic Analysis

The HAV outbreak strains were sequenced to a different extent by the individual countries and hence the overlapping region for all the sequences was too small to distinguish these strains from other HAV strains. Consequently, not all the sequences could be included in the phylogenetic analysis. A region for phylogenetic analysis was carefully selected that could discriminate between strains, covered most of the informative sites among the MSM1-strains, represented both clusters, and included several strains. Particular emphasis was given to phylogenetically informative sites indicated in Figure 1 as they favor some trees over others. A site was considered informative when there were at least two different nucleotides at the site, each of which was present in at least two sequences from different countries. Only isolates with a nucleotide substitution present in




[1] C in this position is present in two of the six isolate i.e. isolate pat22 and pat23

Fig. 1. Overview of HAV MSM1 sequences from outbreaks among homosexual men in European countries within (a) the N-terminal region of VP1 and (b) the VP1/P2A junction of the HAV genome. The main cluster A outbreak strain is used as a consensus. The number of sequenced isolates from the individual countries is given in parenthesis. The 10 informative sites are indicated by arrows and their positions are numbered according to reference strain GBM/WT. The regions used for phylogenetic analysis are indicated with bars.

more than two sequences from each outbreak were included in the sequence alignment and sequence analysis.

The N-terminal VP1 and/or the VP1/P2A regions, corresponding to nt 2,146–2,341 and nt 2,868–3,144 in reference strain GBM/WT, respectively, indicated by bars in Figure 1 were used for phylogenetic analysis. The sequences included in the distance-matrix were of equal length, sequences partially covering these regions were not included in the analysis. The other sequences were HAV strains found in other risk groups in the population in these countries, as well as sequences obtained by BLAST searches in GenBank with the outbreak strains. The sequences were aligned in BioEdit (http://www.mbio.ncsu.edu/BioEdit) and Molecular Evolution Analysis Software (MEGA) version 3.1[Kumar et al., 2004]. The genetic distances were calculated using the Kimura-2-parameter model and the trees were constructed by using neighbor-joining in MEGA. Reproducibility was tested by performing 1,000 bootstraps in MEGA.

### Nucleotide Sequence Accession Numbers

HAV sequences found among homosexual men not included in Figure 2 are obtainable from GenBank; BAV/2/03/DEU (AY656711), 163ADAM (DQ387587), 232ADAM (DQ387654), 235ADAM (DQ387657), 047ADAM/MSM2 (AY343717), 066ADAM/MSM3 (AY343728), 071ADAM/MSM4 (AY343733), pat-21 (DQ469362), pat-22 (DQ469358), pat-23 (DQ469364), pat-24 (DQ469355-6), pat-26 (DQ469352), pat-28 (DQ469365), UK-MSM-04-1–3 (AM260518-20).

## RESULTS

### Strains Involved in Outbreaks Among Homosexual Men

Hepatitis A virus associated with homosexual men in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom over the period 1997–2005 was characterized by sequencing of the N-terminal part of VP1 and/or VP1/P2A regions (Table II). By nucleotide comparison, the majority of strains detected among homosexual men formed a closely related cluster that was named MSM1 (Figs 1 and 2). A few other strains were also detected among homosexual men in some of the countries that were different from the MSM1-strains; the MSM2-MSM4 strains in the Netherlands in 2001–2003 [van Steenbergen et al., 2004], NOR-26 in Norway in 1997–98 [Stene-Johansen et al., 2002], and BCN02 in Spain in 2002 (Fig. 2, Table II). The outbreak strains were of genotype IA, except BCN02 (genotype IB), according to the nomenclature system of Robertson et al. [1992]. The MSM2 and NOR-26 strains were also closely related (data not shown). Phyologenetic analysis of the strains found among homosexual men, among other risk groups in these countries, as well as HAV strains from different geographical regions are shown in Figure 2.

### The MSM1 Outbreak Strains

By nucleotide comparison the MSM1-strains could be subdivided further into two sub-clusters, shown as A and B in Figure 1 and Figure 2. Out of the 10 informative sites indicated (Fig. 1), 7 distinguished between clusters A and B. The HAV sequences from outbreaks in the Netherlands (232ADAM and 163ADAM) in the period 2000–2004, Spain (BCN04.05) in 2003–2004, and Denmark (DK-MSM1-04), Norway (NOR-MSM1-04), and Sweden (SWE-MSM1-04) in 2004 were mainly identical, representing cluster A. The outbreak strain (BCN04.05) from Spain 2003–2004 showed one of the substitutions characteristic for cluster B in the VP1/P2A region. The HAV sequences from homosexual men in the Netherlands in 1997–98 (pat-22) and 2003–2004 (235ADAM), Norway in 1997–98 (NOR-25), the United Kingdom (UK-MSM-04-01 and UK-MSM-04-3) in 2004, Sweden (SWE-29/04) in 2004, Spain in 2004–2005 (BCN05.04), and Germany in 2002–2003 (BAV/2/03/DEU) formed cluster B. The NOR-25 outbreak strain from Norway in 1997–98 contained five of six characteristic sites of cluster B in the VP1/P2A region, but only one of the three sites that were covered in the N-terminal of VP1. The outbreak strains; NOR-25, UK-MSM-04-1, BCN04.05, BAV/2/03-DEU, and 232ADAM showed substitutions characteristic for these particular outbreak strains in addition to the common pattern of the clusters. A single substitution in addition to the cluster B pattern was common to one of the Swedish strains (SWE-29/04) and one of the Spanish outbreaks strains (BCN05.04). The strains from the Netherlands (pat-22 and pat-23) and Norway in 1997–98 (NOR-25) showed a common unique substitution.

### Characterization of MSM1-Related Outbreaks

An epidemic curve of cases of hepatitis A associated with the MSM1-strains classified by the three case definitions (Table II) for the period 1997–2005 from the seven European countries is shown in Figure 3. The total number of MSM1-related cases is shown on a country basis for the period September 2003 to August 2005 in Figure 4.

## DISCUSSION

Several outbreaks of hepatitis A have been reported among homosexual men in low endemicity regions in Europe during the last decade. The dissemination of HAV among homosexual men in European countries was therefore studied by molecular epidemiology including samples from Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom. These countries were all affected by outbreaks in the homosexual communities in recent years. The majority of these strains formed a cluster of similar HAV strains that were named MSM1 (Figs. 1 and 2). Very few nucleotide substitutions were observed within the cluster of MSM1-strains (Fig. 1). The results of this study suggest that highly similar HAV strains are exchanged among the

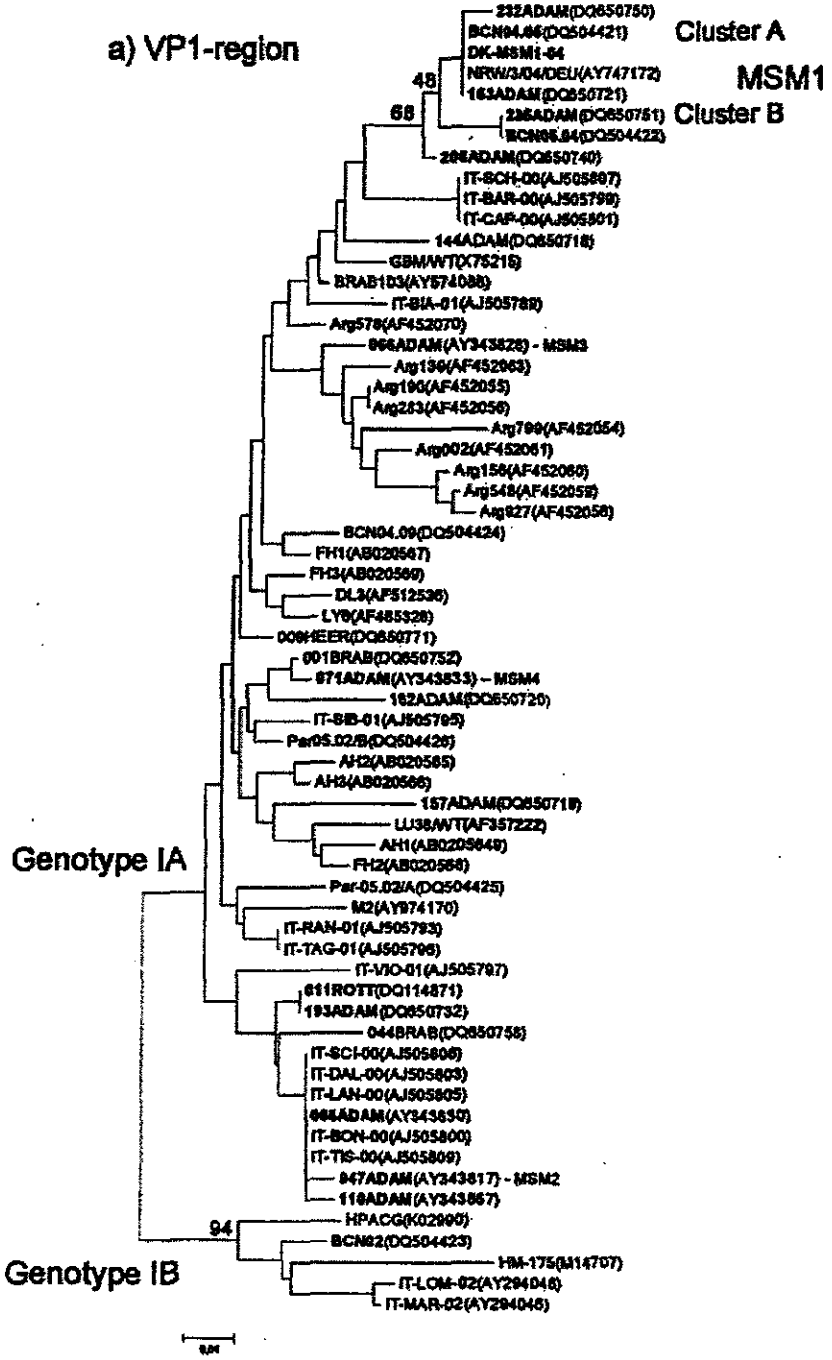



Fig. 2. Phylogenetic trees of the N-terminal of VP1 and the VP1/P2A regions of HAV outbreak strains found among homosexual men in bold in comparison with strains found in other risk groups and strains from different geographical regions. The phylogenetic analysis is based upon the N-terminal part of VP1 and the VP1/P2A junction corresponding to nt 2,146–2,341 and nt 2,868–3,144 in reference strain GBM/WT respectively. Bootstrap values (1,000 replicas) for the clusters of MSM1 are shown. The trees were constructed by use of neighbor-joining of Kimura-2 parameter corrected distances. Accession numbers are shown in parenthesis.

homosexual communities in Europe. HAV is a single-stranded RNA virus that undergoes rapid genetic changes due to the RNA-dependent RNA polymerase that replicates with low fidelity and lack of proofreading activity [Sanchez et al., 2003a]. The nucleotide variation seen between the MSM1-strains were probably caused by natural drift in the HAV population that appears with time, and hence that the MSM1 strains were of the same origin. The circulation of similar HAV strains in the homosexual population on an international basis over

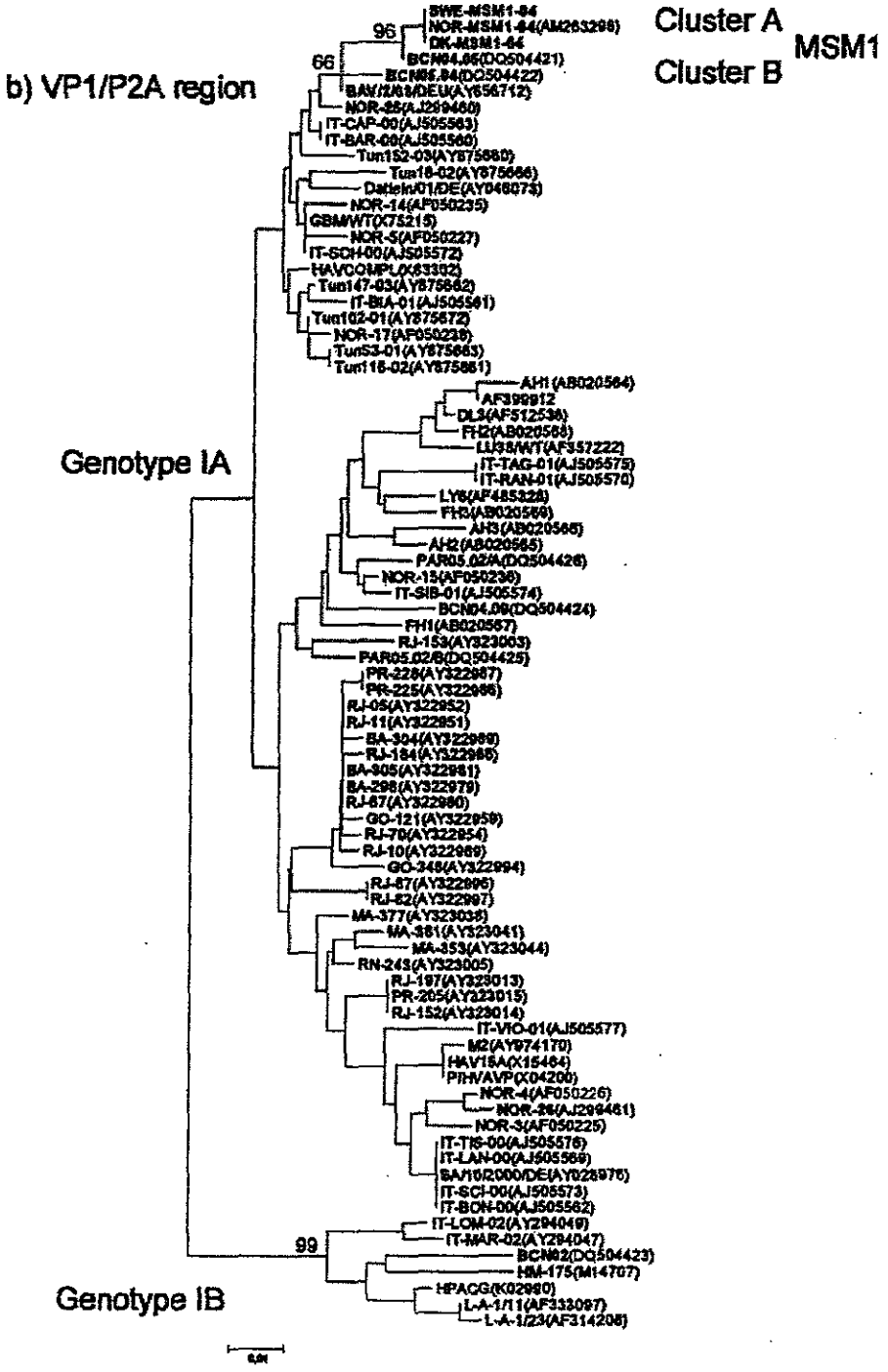



Fig. 2. (*Continued*)

time suggests an endemic HAV population among homosexual men.

The clustering of MSM1-strains was similar for the two regions (N-terminal of VP1 and VP1/P2A), except for the NOR-25 strain from Norway in 1997–98 (Fig. 1a, b). Strain NOR-25 segregated in cluster B in the VP1/P2A junction, whereas it shared two common informative sites with cluster A and one common site with cluster B in the N-terminal region of VP1. Also the bootstrap values could not clearly support clustering of this strain with the other MSM1-strains (data not shown). It is disputable whether this strain should be regarded as an MSM1-strain. The fact that this strain shares the same substitutions as MSM1-related strains from the same

TABLE II. Cases of Hepatitis A Associated With Homosexual Men in Seven European Countries Classified by Case definition in the Period January 1997 to August 2005

| Country | Strain | Confirmed cases | Probable cases | Possible cases | Total | Outbreak period (mm.yyyy) |
|---|---|---|---|---|---|---|
| Denmark | MSM1 | 19 | 21 | 95 | 135 | 01.2004–12.2004 |
| Germany | MSM1 | 9 | 9 | 59 | 77 | 11.2002–07.2003 |
| The Netherlands | MSM1 | 6 | | | 6 | 01.1997–03.1998 |
| | MSM1 | 53 | 7 | | 60 | 08.2000–10.2004 |
| | MSM2 | 34 | 6 | | 40 | 01.2001–02.2004 |
| | MSM3 | 3 | | | 3 | 05.2001–08.2001 |
| | MSM4 | 1 | 2 | | 3 | 06.2001–08.2001 |
| Norway | MSM1 | 12 | | 9* | 21 | 10.1997–03.1998 |
| | NOR-26 | 5 | | | 5 | 10.1997–03.1998 |
| | MSM1 | 48 | 7 | 25 | 80 | 05.2004–12.2004 |
| Spain | BCN02 | 1 | | 37 | 38 | 01.2002–11.2002 |
| | MSM1 | 2 | | 70 | 72 | 07.2003–06.2004 |
| | MSM1 | 2 | | 6 | 8 | 12.2004–08.2005 |
| Sweden | MSM1 | 14 | 6 | 15 | 35 | 01.2004–09.2004 |
| United Kingdom | MSM1 | 10 | 4 | 3 | 17 | 09.2004–11.2004 |
| Total | | 219 | 63 | 319 | 601 | |

*Possible cases could be caused by either of the two co-circulating strains (MSM1 and NOR-26) in 1997–98 in Norway.

time, isolated in the Netherlands (Fig. 1b), supports strongly the notion that these strains are of the same origin. These strains are several years older and might be regarded as ancestral strains or they evolved from the same ancestral strain resulting in evolution of two slightly different clusters. A recombination event in one of the lineages can not be discounted. The outbreak in the United Kingdom involved two different MSM1 strains (Fig. 1b). The major outbreak strain (UK-MSM-04-1) appears to be more distinct than the other MSM1 strains as it differs by two nucleotides from the consensus sequence of cluster B resulting in an amino acid substitution.

An endemic hepatitis A situation requires a continual circulation of strains that are maintained in large populations by continual supply of non-immunized persons. Molecular epidemiological studies show that in high endemic regions there are circulating populations of homologous HAV strains, whereas in low endemic regions strains are highly divergent due to importation from different regions where high endemic transmission occurs [Robertson et al., 1992]. In the homosexual communities across Europe, homologous

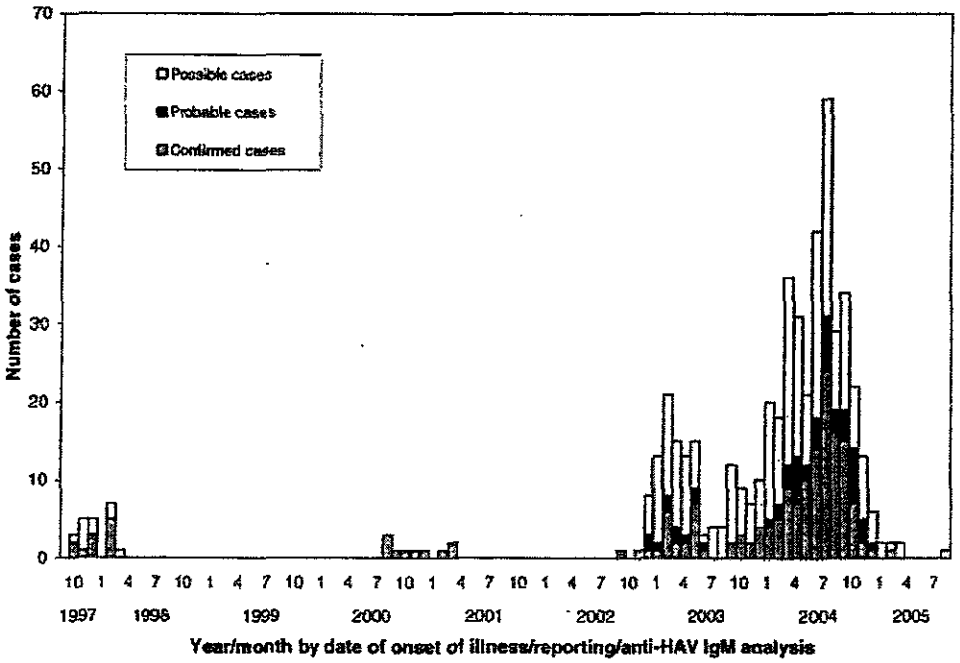



Fig. 3. Epidemic curve of cases of hepatitis A in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom from September 1997 to August 2005 classified by case definition. The cases are indicated by month of onset of illness for Denmark, Germany, Norway, and Sweden, month of reporting for the Netherlands, month of testing anti-HAV IgM for the United Kingdom and Spain.

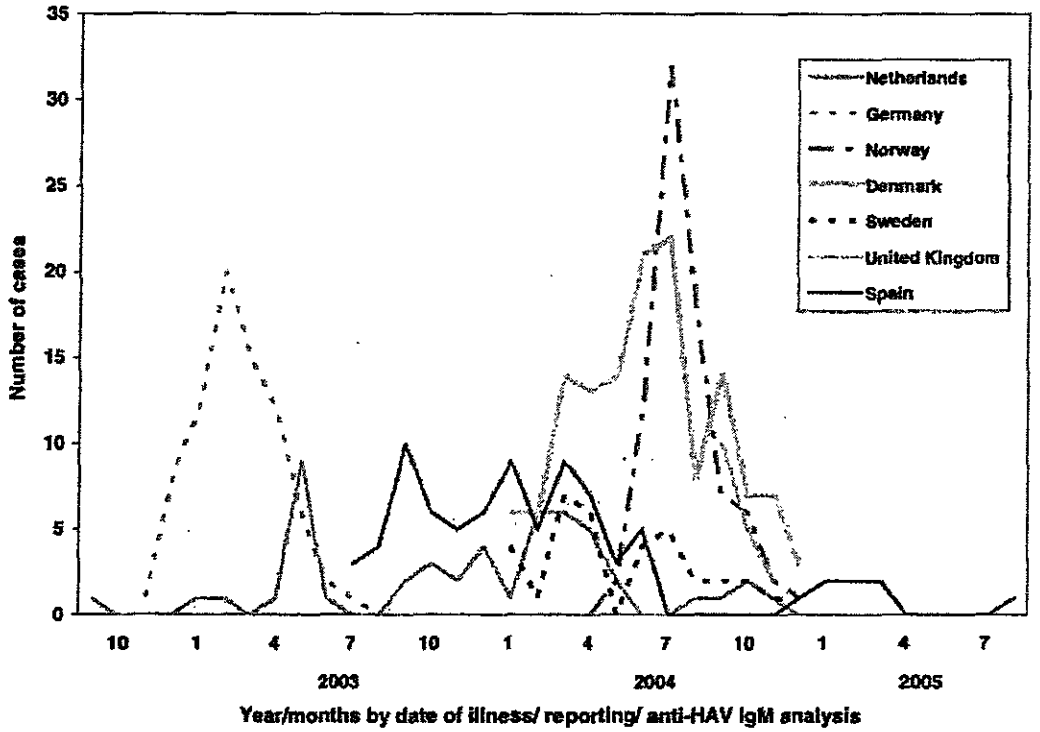



Fig. 4. Cases of hepatitis A associated with the MSM1-outbreak strain in Denmark, Germany, the Netherlands, Norway, Spain, Sweden, and the United Kingdom during the period September 2003 to August 2005. Recorded data as in Figure 3.

strains were found from 1997 until 2004, suggesting interconnected endemic populations of HAV in these communities. In the Netherlands, the strains found among homosexual men appeared as sporadic cases from 2000 to 2004. The detection of MSM1-strains among homosexual men in Norway and the Netherlands in 1997–1998 indicates that this strain has been circulating among homosexual men for a long time. However, no outbreaks were observed in 1998–2000 and 2001–2002 (Fig. 3). Reported cases might have remained unrecognized as homosexual men since not all countries in Europe include sexual orientation in their surveillance data. Also, previously reported outbreaks among homosexual men in London, Berlin, and Paris were not included in the study because no samples could be obtained. Alternatively, the appearance of sporadic closely related strains over time might be due to several independent introductions of HAV strains of the same geographical origin to the homosexual communities in Europe, but this seems less likely.

The homosexual communities within the individual countries in Europe are probably too small to maintain HAV in their population over time due to a limited number of susceptible individuals. Following outbreaks, small communities develop herd immunity that gradually will decline with the recruitment of non-immunized individuals to the communities [Widell et al., 1982, 1983; Gust, 1992; Melnick, 1995]. Consequently, in smaller communities, outbreaks may occur with a cyclic appearance. Seen on a more global basis, the homosexual communities across Europe are probably so large and interconnected that HAV may circulate for years resulting in an endemic situation among homosexual men. Notified cases of HAV in these European countries are mainly seen in certain risk groups whose habits or lifestyle put them at high risk of infection, and not in the general population. Other distinct strains were detected among travellers to endemic regions and intravenous drug users [Grinde et al., 1997; Stene-Johansen et al., 1999, 2002; Bruisten et al., 2001; van Steenbergen et al., 2004; Sunqvist et al., 2005; Tjon et al., 2005b] suggesting that the MSM-related characterized strains in this study were found among homosexual men exclusively.

The molecular data suggest transmission of HAV among men in homosexual communities in the European countries. The epidemiological data alone could only indicate transmission between homosexual men in the different European countries to a very small extent [Mazick et al., 2005] emphasizing the need of molecular epidemiology in outbreak investigations. The sexual practices among homosexual men place them at high risk of infection, and increased travel with visits to gay clubs contribute to a wide spread of HAV among homosexual men in Europe. HAV infection seems to be endemic in this risk group and new outbreaks among homosexual men may be expected soon. Vaccination of homosexual active men in Europe is therefore necessary

to prevent further outbreaks in these communities. A local vaccination policy based upon distribution of vaccine to homosexual active men during outbreaks may provide only limited preventive measures when HAV spreads on a global basis.

## ACKNOWLEDGMENTS

We acknowledge the collaboration of Helena Pañella from the Barcelona Public Health Agency and Margarita Salvadó from the Catalonia Reference Laboratory for providing the Spanish samples to the Enteric Virus Group of the University of Barcelona. We further thank Michael Howitz and Kåre Mølbak from Statens Serum Institute, Department of Epidemiology, Copenhagen. Johannes Schnitzler, fellow of the field epidemiology training programme (FETP) in Germany at that time and now at WHO, Geneva and Christina Frank, Robert Koch-Institute, for the participation in the outbreak investigation.

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2007年2月7日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | BSE confirmed in Alberta.<br>Canadian Food Inspection Agency<br>website:<br>www.inspection.gc.ca/english/toce.shtml | 公表国<br>カナダ | |
| 販売名(企業名) | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| カナダ食品検査庁によって発表された本報告書は,アルバータ州で見つかった9例目のウシ海綿状脳症(BSE)症例に関するものである。当該雄ウシは2000年に生まれ，死亡時は約6.5歳であった。また，発見された農場にて出生・生育した。カナダで反芻動物由来の飼料禁止が実施された1997年以後の出生であるため，当該ウシは，おそらく非常に少量の感染物質に，おそらく生後1年以内に曝露したことが示唆される。当該ウシの飼料が何であったかを調査し，当時の同じ群の他のウシを追跡するために，詳細な調査が進行中である。当該ウシは，これまでのカナダにおける全BSE症例と同様，全国監視プログラムによって同定された。このプログラムでは2003年に北米で最初のBSE症例が出現して以来，高リスクのウシ150,000頭を検査してきた。これらのデータから，実際にはカナダにおけるBSEの発現率は非常に低く，飼料禁止の強化が2007年7月12日に発効されることからさらに低くなることが期待される。 | BYL-2007-0271 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| カナダで9例目のBSE症例は,高リスクのウシ150,000頭の中から体系的な調査によって同定された。この新規症例が報告されたが,カナダにおけるBSEリスクは非常に低いという結論は変わらない。 | 現時点で新たな安全対策上の措置を講じる必要はないと考える。 |

Canadian Food Inspection Agency / Agence canadienne d'inspection des aliments

BYL-2007-0

# BSE CONFIRMED IN ALBERTA

OTTAWA, February 7, 2007 - The Canadian Food Inspection Agency (CFIA) has confirmed the diagnosis of bovine spongiform encephalopathy (BSE) in a mature bull from Alberta. The animal's carcass is under CFIA control, and no part of it entered the human food or animal feed systems.

Preliminary information indicates that the age of the animal falls well within the age range of previous cases detected in Canada under the national BSE surveillance program. This signifies that the animal was exposed to a very small amount of infective material, most likely during its first year of life.

An epidemiological investigation directed by international guidelines is underway to examine what the animal was fed early in its life and to identify its herdmates at the time. All findings will be publicly released once the investigation concludes.

Under Canada's enhanced feed ban, which comes into effect on July 12, 2007, BSE should be eliminated from the national cattle herd within approximately 10 years. The CFIA expects the periodic detection of a limited number of cases to continue as the level of BSE continues to decline.

The finding of a mature animal should not impact Canada's BSE country categorization submission to the World Organization for Animal Health (OIE). The science-based BSE risk-level determination process requires that a country is able to demonstrate a full understanding of the pathways that resulted in BSE exposure and expression, as well as the implementation of appropriate comprehensive measures to block those pathways and protect human and animal health, leading to the eradication of the disease over time.

The animal was identified at the farm level by the national surveillance program, which has detected all cases found in Canada. The program targets the highest risk cattle populations and has tested roughly 150,000 animals since 2003. The surveillance results reflect an extremely low incidence of BSE in Canada.

- 30 -

For information:
Canadian Food Inspection Agency
Media relations: 613-228-6682

Date Modified: 2007-02-07

Canada

# 医薬品
# 医薬部外品　研究報告　調査報告書
# 化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2007 年 1 月 12 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | FDA proposes barring certain cattle material from medical products as BSE Safeguard.<br>FDA News,<br>11 January 2007 | 公表国<br>米国 | |
| 販売名(企業名) | | | | | | |

**研究報告の概要**

ウシ海綿状脳症(BSE)の原因となる物質のヒトへの伝播リスクをさらに減少させるために，米国食品医薬品局(FDA)はヒトへの使用を目的とした一部の医薬品(薬剤，ワクチン及び医療機器)ならびに反芻動物への使用を目的とした薬剤でのウシ由来原料の使用を制限することを提案した。この新しい規制では，罹患ウシにおいて最高濃度の BSE 因子を含むことが確認されている部位すべてと，以下のものが禁止される：

・30 ヵ月齢以上のウシの脳，頭蓋，眼及び脊髄
・月齢又は健康状態を問わず，全てのウシの扁桃腺及び小腸の一部
・歩行不能な「ダウナー」牛の全部位
・食用の検査を受けていないウシ，及び合格していないウシの全部位
・本規則案での禁止部位による汚染を防ぐための適切な処置を行っていないウシ胎仔血清
・本規則案での禁止部位由来の獣脂で，0.15%を超える不溶性不純物を含有する場合
・機械によって加工された牛肉

確実にこれらの禁止事項を企業に従わせるため，FDAは医薬品の原料として，あるいは製造工程の一部として使用されるウシの全部位が規制要件を満たすことを明示する記録の保管要求を提案している。

**使用上の注意記載状況・その他参考事項等**

BYL-2007-0268

www.fda.gov/bbs/topics/NEWS/2007/NEW01545.html

| 報告企業の意見 | 今後の対応 |
|---|---|
| 1997 年以降，BSE リスクを低減するために FDA は厳重な措置(例えば，反芻動物由来飼料の使用禁止)を取っている。これまでの BSE 報告件数がわずかであることから，事実上のリスクは非常に小さく，これらの措置は有効であると考えられる。 | 現時点で新たな安全対策上の措置を講じる必要はないと考える。 |

## FDA News

**FOR IMMEDIATE RELEASE**
P07-04
January 11, 2007

**Media Inquiries:**
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# FDA Proposes Barring Certain Cattle Material From Medical Products As BSE Safeguard

The U.S. Food and Drug Administration is proposing to limit the materials used in some medical products in order to keep them free of the agent thought to cause mad cow disease, also known as bovine spongiform encephalopathy or BSE.

This is the latest in a series of BSE safeguards that would bar material that has been found to harbor the highest concentrations of this fatal agent in infected cattle. These materials would be prohibited from use as ingredients in medical products or elements of product manufacturing.

The proposed rule would cover drugs (prescription, over-the-counter, and homeopathic), biologics (such as vaccines) and medical devices intended for use in humans as well as drugs intended for use in ruminant animals like cattle and sheep. Cattle can get mad cow disease, while sheep can get a similar disease known as scrapie.

"These measures build on a series of barriers FDA and the U.S. Department of Agriculture have erected to further protect humans from exposure to the fatal agent linked to BSE," said Andrew von Eschenbach, M.D., Commissioner Food and Drugs. "This proposed rule adds one more safeguard that will reduce the risk of transmission even further."

The cattle materials prohibited in the proposed rule are those that pose the highest risk of containing infectious material and include:

- the brain, skull, eyes and spinal cords from cattle 30 months and older;
- the tonsils and a portion of the small intestines from all cattle regardless of their age or health;
- any material from "downer" cattle–those that cannot walk;
- any material from cattle not inspected and passed for human consumption;
- fetal calf serum if appropriate procedures have not been followed to prevent its contamination with materials prohibited by this proposed rule;
- tallow that contains more than 0.15 percent insoluble impurities if the tallow is derived from materials prohibited by this proposed rule and;
- mechanically separated beef.

To ensure that companies comply with these prohibitions, FDA proposes to require that records be kept to demonstrate that any cattle material used as an ingredient in these medical products or as part of their manufacturing process meet the rule's requirements.

Since 1996, strong evidence has accumulated for a causal relationship between ongoing outbreaks of mad cow disease in Europe and a disease in humans called variant Creutzfeldt-Jakob (vCJD) disease. Both disorders, which are thought to be caused by an unconventional transmissible agent, are invariably fatal brain diseases with incubation periods typically measured in years. Transmission of the BSE agent to humans, leading to vCJD, is believed to occur via ingestion of cattle products contaminated with the BSE agent; however the specific products associated with this transmission are unknown.

About 200 cases of vCJD have been identified worldwide, including three cases in the U.S. However, there is no evidence that those three patients contracted the BSE agent in the U.S.

FDA and USDA's efforts to help protect the public from vCJD have included several other significant steps such as the FDA's 1997 ruminant feed regulation, which forbids the use of certain mammalian-origin proteins in ruminant feed. Also, a 2005 interim final rule bans the use of certain high-risk cattle material in food, dietary supplements and cosmetics.

####

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 11 月 1 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Isolation from cattle of a prion strain distinct from that causing bovine spongiform encephalopathy.<br>Béringue, V. et al.,<br>PLoS Pathog., 2, 956-963 (2006) | 公表国<br>フランス | |
| 販売名 (企業名) | | | | | | |

**研究報告の概要**

ヨーロッパにおいて伝染性海綿状脳症 (TSEs) のために死亡及び屠殺した畜牛の大規模な検査が行われた結果，電気泳動上 BSE 因子の PrP とは異なる，異型 PrP ($PrP^{res}$) プリオンタンパク (H 型と呼ばれる) が発見された。フランスの畜牛から分離された 7 種のプリオンタンパクをトランスジェニックマウス (Tg) マウスへ脳内接種し比較することにより，H 型 $PrP^{res}$ の伝播性を評価した。マウスには，ウシ (tgBov) 及びヒツジ (tgOv) PrP のいずれかを発現させるようにした (本系統ではマウス PrP は発現していない)。全てのマウスで神経症状が発症し，tgBov Tg マウスは 400 日の間に，tgOv Tg マウスは 600 日の間に海綿状脳症で死亡した。次世代マウスでは，平均生存時間は 100 日間まで減少した。ウシ海綿状脳症 (BSE) または異型クロイツフェルト・ヤコブ病 (vCJD) 因子に感染すると，$PrP^{res}$ は tgOv Tg マウスの脾臓内でウエスタンブロット法で検出可能なレベルまで蓄積した。一方，H 型プリオンを接種した tgOv Tg マウスでは，脾臓内に $PrP^{res}$ は検出されなかった。罹患したマウスの脳切片の分析によると，H 型 $PrP^{res}$ と BSE $PrP^{res}$ の構造上の一致は一部分のみで，神経解剖学的には異なる病変が判明した。さらに，H 型プリオン感染マウスでは重度の海綿状態が観察された。イタリアのヒツジのスクレピー分離株で行われた同様の実験によって，現時点では H 型プリオンは tgOv Tg マウスに接種した全ての TSE 分離株と関連性がなく，したがってこれは新型のプリオンであるという結論になった。
著者らは複数のプリオン株の存在，あるいはいくつかの動物においてプリオンが分岐進化している可能性を示唆した。しかしながら，その起源とヒトの健康への潜在的リスクは今日現在，未知のままである。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本調査結果から，TSE に関与するプリオンは当初考えられていた以上に多様性に富み，またヒトへの感染はプリオンの種類によって異なることが示唆された。これは，ウシ由来製品を含有する血漿分画製剤の製造工程におけるプリオン除去工程を評価する際に考慮が必要となる可能性がある。 | 弊社の血漿分画製剤の製造工程において，これまでに同定されている種々のウシプリオンに対してプリオン除去工程は効果的であることが確認されている。<br>現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

BYL-2007-0252



57

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# Isolation from Cattle of a Prion Strain Distinct from That Causing Bovine Spongiform Encephalopathy

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**To date, bovine spongiform encephalopathy (BSE) and its human counterpart, variant Creutzfeldt-Jakob disease, have been associated with a single prion strain. This strain is characterised by a unique and remarkably stable biochemical profile of abnormal protease-resistant prion protein ($PrP^{res}$) isolated from brains of affected animals or humans. However, alternate $PrP^{res}$ signatures in cattle have recently been discovered through large-scale screening. To test whether these also represent separate prion strains, we inoculated French cattle isolates characterised by a $PrP^{res}$ of higher apparent molecular mass—called H-type—into transgenic mice expressing bovine or ovine PrP. All mice developed neurological symptoms and succumbed to these isolates, showing that these represent a novel strain of infectious prions. Importantly, this agent exhibited strain-specific features clearly distinct from that of BSE agent inoculated to the same mice, which were retained on further passage. Moreover, it also differed from all sheep scrapie isolates passaged so far in ovine PrP-expressing mice. Our findings therefore raise the possibility that either various prion strains may exist in cattle, or that the BSE agent has undergone divergent evolution in some animals.**

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## Introduction

While transmissible spongiform encephalopathies (TSEs) in small ruminants and humans are believed to involve distinct prion strains [1,2], a single prion strain has been associated so far with bovine spongiform encephalopathy (BSE) and its human counterpart, variant Creutzfeldt-Jakob disease (vCJD) [3–6]. In particular, the abnormal, protease-resistant form of prion protein ($PrP^{res}$) that accumulates in the brains of infected individuals [7] shows a consistently unique electrophoretic profile in immunoblots [8]. However, the biochemical testing of the brains of slaughtered and fallen cattle, which was intensified since 2000 in European countries as a means to protect the consumers, has led to the discovery of positive samples that showed distinct $PrP^{res}$ profiles. These atypical profiles have been sorted into two groups so far, provisionally termed H-type when the size of the protease resistant fragments is higher than for BSE, and bovine amyloidotic spongiform encephalopathy, or L-type, when it is lower [9,10]. These observations raise the possibility that as yet unrecognised prion strains may exist in cattle as in other species [11], and have potential implications in terms of public health. Unlike bovine amyloidotic spongiform encephalopathy isolates, which derive from animals with defined histopathological abnormalities [10], precise information corroborating a prion disease is lacking for H-type cases. It was therefore crucial to determine through experimental transmission whether such cases reflect some alteration in PrP metabolism, possibly in aging animals, or involve a truly infectious agent.

In this study, we report the transmission of a TSE-like disease by inoculation of French cattle isolates identified as H-type variants to two lines of PrP transgenic mice. Furthermore, we provide compelling evidence that this agent has unique features compared to epizootic BSE and other related agents. We also establish that there is no link with ovine TSE isolates transmitted so far to these models.

## Results

### H-Type Isolates Are Transmissible to Mice

Two transgenic mouse lines were used as recipient for transmission experiments. The tg540 line is a newly established line that expresses bovine PrP (Protocol S1), resulting in an enhanced susceptibility to BSE agent compared to conventional mice [6,12]. The tg338 line, expressing the VRQ ($Val^{136}Arg^{154}Gln^{171}$) allele of ovine PrP, has allowed an efficient transmission of natural scrapie isolates from sheep and goat [13,14]. The rationale for including tg338 mice in this study was the possibility that characterisation of a prion

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**Abbreviations:** BSE, bovine spongiform encephalopathy; $PrP^{res}$, protease-resistant prion protein; TgBov, bovine tg540; TgOv, ovine tg338; TSE, transmissible spongiform encephalopathies; vCJD, variant Creutzfeldt-Jakob disease

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## Synopsis

Prions are unconventional agents of proteic nature that are formed of abnormal conformations of the host-encoded prion protein (PrP). They cause fatal neurodegenerative diseases in both animals and humans, and can be transmitted between species as exemplified in humans by the emergence of variant Creutzfeldt-Jakob disease following the epidemic of bovine spongiform encephalopathy (BSE) in the United Kingdom. Since diagnosis of prion infection is only possible once the central nervous system has been invaded, brains of slaughtered or fallen cattle are routinely screened in Europe to protect the consumers from BSE. This has unexpectedly led to the discovery of unprecedented PrP conformations that were distinct from the single one associated so far with BSE or BSE-related diseases. To precisely determine their etiology, the authors have studied the transmissibility of these new conformations, termed H-type, to transgenic mice expressing either bovine or ovine PrP. They show that these cases are highly pathogenic for these mice. The authors also demonstrate that they are not directly related to the agent involved in the BSE epidemic, supporting the view for isolation of a new prion strain from cattle, whose prevalence and associated zoonotic risk should be carefully monitored in the future.

accidentally passed from small ruminants to cattle might be facilitated on such mice, by comparison with the ovine isolates transmitted so far. Tg540 (tgBov) and tg338 (tgOv) mice overexpress PrP in the brain at similar levels (~8- to 10-fold). Both lines have a normal lifespan, the same as $PrP^{0/0}$ mice on which the transgenes were introduced. H-type isolates representative of a series of seven samples identified in France were inoculated intracerebrally to tgBov and tgOv mice (Table 1). Typical BSE agents from cattle and from other species was inoculated to the same mice for the sake of comparison. Remarkably, all H-type isolates induced a neurological disease on primary transmission, with a 100% attack rate in both mouse lines. The mean survival times observed with cases no. 1 and no. 2 in tgBov mice, ~400 d, appeared to be prolonged compared to those for cattle, sheep, goat BSE, and human vCJD inocula, which ranged from ~250 to 380 d. Such a discrepancy could reflect a lesser infectivity of H-type samples, consistent with their comparatively lower $PrP^{res}$ content [9]. Moreover, the survival time was reduced by ~100 d on subpassage, approaching that for BSE from cattle or other sources on secondary passage, or on primary passage for inocula of presumably higher titre (i.e., producing no substantial reduction of survival time on subpassage: BSE no. 3 and ARQ [$Ala^{136}Arg^{154}Gln^{171}$] no. 1). Upon transmission to tgOv mice, the mean incubation period produced by the four H-type cases was strikingly homogeneous (586–612 d), consistent with a potentially unique agent (Table 1). This was comparable to or even shorter than the incubation periods of epizootic BSE or related inocula on the same mice (560–792 d). As illustrated in Figure 1, the relative incubation periods observed on tgOv and tgBov mice appeared to differ significantly among the H-type and BSE-type agents. In addition, the reduction in incubation period observed upon secondary transmission of H-type (case no. 2) on tgOv mice was significantly less dramatic than that observed for vCJD and sheep BSE inocula (Table 1). Overall, these suggested that H-type and BSE might be different TSE agents.

### H-Type $PrP^{res}$ Profile Is Preserved in Transgenic Mice

The brains of diseased mice were analysed by immunoblotting for the accumulation of abnormal PrP. $PrP^{res}$ was readily detected in all mice tested since the first passage, consistent with the efficient transmission observed in both lines (10/10 and 33/33 positive brains for tgBov and tgOv mice, respectively). The $PrP^{res}$ molecular profile was fairly uniform

**Table 1.** Transmission of Bovine Molecular Variant Cases (H-Type) to Transgenic Mice Expressing Bovine or Ovine PrP

| Isolate | Case Number | Passage | Mean survival time, d ± SEM ($n/n_0$)[a] | |
|---|---|---|---|---|
| | | | tgBov Mice | tgOv Mice |
| H-type | 1 | First | 414 ± 10 (5/5) | 612 ± 26 (10/10) |
| | | Second | 317 ± 6 (8/8) | ND |
| | 2 | First | 401 ± 9 (5/5) | 595 ± 18 (8/8) |
| | | Second | 296 ± 3 (9/9) | 319 ± 10 (6/6) |
| | 3 | First | ND | 607 ± 12 (6/6) |
| | 5 | First | ND | 586 ± 15 (9/9) |
| BSE | 1 | First | 377 ± 22 (6/6) | ND |
| | 3 | First | 298 ± 7 (9/9) | 704 ± 36 (7/7) |
| | | Second | 283 ± 10 (5/5) | NA |
| Sheep BSE[b] | ARQ 1 | First | 278 ± 2 (6/6) | 560 ± 60 (5/5) |
| | | Second | 263 ± 6 (6/6) | 178 ± 2 (4/4) |
| | ARQ 3 | First | 339 ± 25 (5/5) | ND |
| | ARR 1 | First | 340 ± 8 (7/7) | ND |
| Goat BSE[c] | CH636 | First | 253 ± 9 (6/6) | 590 ± 43 (4/4) |
| | | Second | 291 ± 27 (5/5) | NA |
| Variant CJD | NHBY0/0003 | First | 343 ± 8 (5/5) | 792 ± 22 (6/6) |
| | | Second | 293 ± 11 (6/6) | 195 ± 9 (6/6) |
| Control | Sheep brain | First | 793 ± 26 (0/9) | 835 ± 15 (0/6) |

NA, not available; ND, not done.
[a]Intracerebral inoculation with 2 mg brain tissue equivalent; $n/n_0$: diseased/inoculated.
[b]Experimental cases.
[c]Field case.
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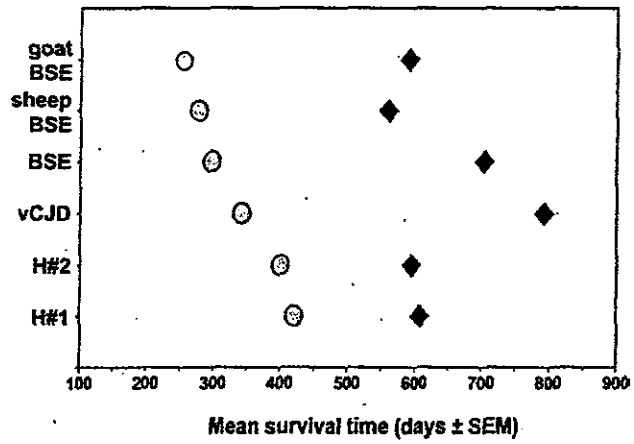


**Figure 1.** Survival Time in Transgenic Mice Infected with H-Type and BSE-Type Agents

Mean survival times (days ± SEM) upon primary transmission are shown for tgBov (grey circles) and tgOv (black diamonds) mice inoculated with H-type cases, BSE, and related isolates (see Table 1). The intervals between the incubation times on each line are significantly different for H-type and BSE agents ($p < 0.0002$, Fisher test).

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among the isolates. Remarkably, like the BSE agent for which the typical signature was conserved whatever the donor species (≥ 3 brains analysed per combination), the H-type agent essentially retained its biochemical phenotype upon serial transmission to tgBov as well as to tgOv mice expressing a heterologous $PrP^C$ (Figure 2 and below). Compared to BSE $PrP^{res}$, it was characterised by a significantly higher apparent molecular mass (difference measured for unglycosylated species: 0.9 ± 0.05 kDa and 0.7 ± 0.06 kDa in tgBov and tgOv mice, respectively) and the relative proportions of glycoforms were essentially similar. A further difference was the lack of detectable $PrP^{res}$ in the spleen of H-type diseased tgOv mice (three to five spleens tested per isolate), while this accumulated at substantial levels after BSE or vCJD infection (Figure 2B).

## H-Type and Epizootic BSE Agents Exhibit Distinct Neuropathological Features

We next examined the $PrP^{res}$ targeting and the vacuolation in the brain, which are known to exhibit a strain-dependent variation [6,15,16]. This was performed on tgBov mice since they express a $PrP^C$ homologous to that of the donors, including the number of octarepeats [17], thus providing a relevant context for comparing H-type and epizootic BSE isolates. H-type isolates showed a similar distribution of $PrP^{res}$ deposits on both primary and

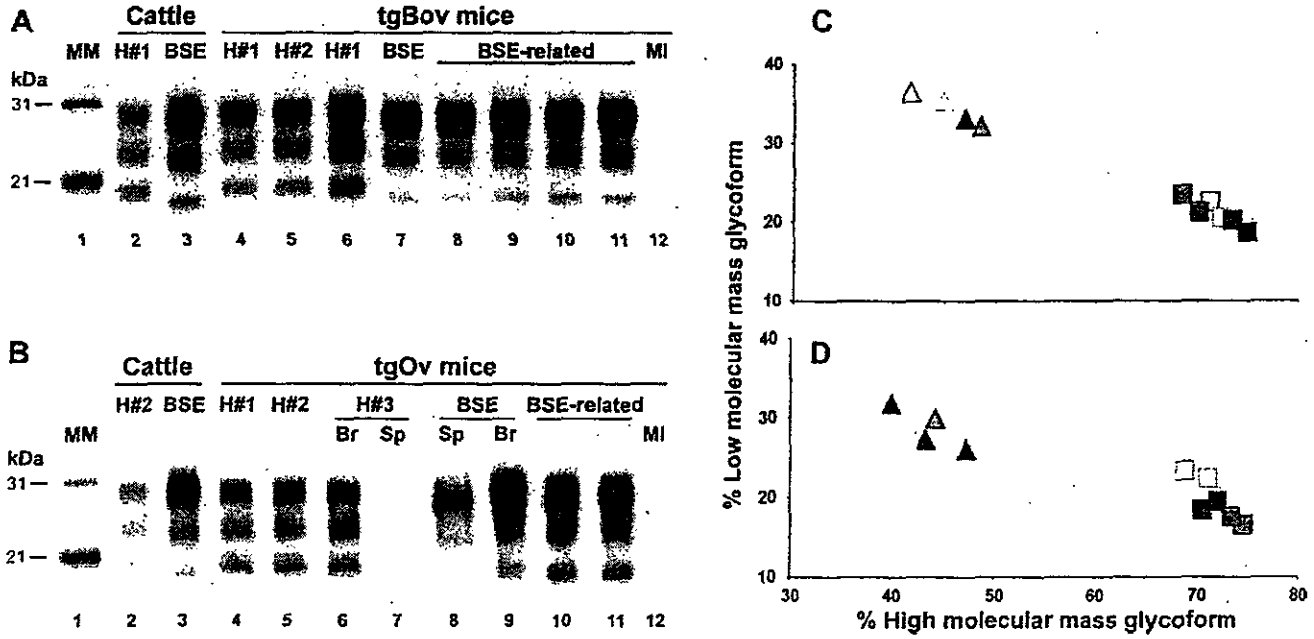


**Figure 2.** Western Blot Analysis of $PrP^{res}$ in the Brains of Transgenic Mice Infected with H-Type or BSE-Type Agents

(A) Primary or secondary (lane 6) transmission to tgBov mice. BSE-type inocula include cattle BSE no. 3 (lanes 3 and 7), sheep BSE ARQ no. 1 and ARR no. 1 (lanes 8 and 9), goat BSE (lane 10), and vCJD (lane 11). The $PrP^{res}$ profiles of both H-type and BSE agents in cattle (lanes 2 and 3) are essentially similar to those in tgBov mice (lanes 4–11). Brain tissue equivalent loaded: 2.5 mg in lane 2; 0.15 mg in lane 3; 0.5 mg in lanes 4–12. MI, mock-infected brain; MM, molecular markers.

(B) Primary transmission to tgOv mice. H-type agent shows a distinct $PrP^{res}$ pattern in the brain (Br) compared to BSE agents (lane 9, BSE no. 3; lane 10, goat BSE; lane 11, vCJD). Note the lack of $PrP^{res}$ signal in the spleen (Sp) of H-type–infected mice (lane 7), unlike that in BSE-infected mice (lane 8). Brain or spleen tissue equivalent loaded: 3 mg in lane 2; 0.15 mg in lane 3; 0.5 mg in lanes 4–6; 2 mg in lanes 7–12.

(C and D) Ratio of high- and low-molecular-mass $PrP^{res}$ glycoforms in the brains of tgBov (C) and tgOv (D) mice following challenge with H-type or BSE agents (data plotted as means ± SEM). H-type isolates are represented as triangles (no. 1, blue; no. 2, orange; no. 3, pink; and no. 5, black) and BSE agents as squares (BSE no. 3, red; sheep BSE ARQ no. 1, grey; sheep BSE ARR no. 1, yellow; goat BSE, brown; and vCJD, light blue). Secondary transmissions are represented by unfilled symbols of the same colour. Note the strikingly distinct glycoform ratio between H-type and BSE groups in both mouse lines, as reported in cattle [9].

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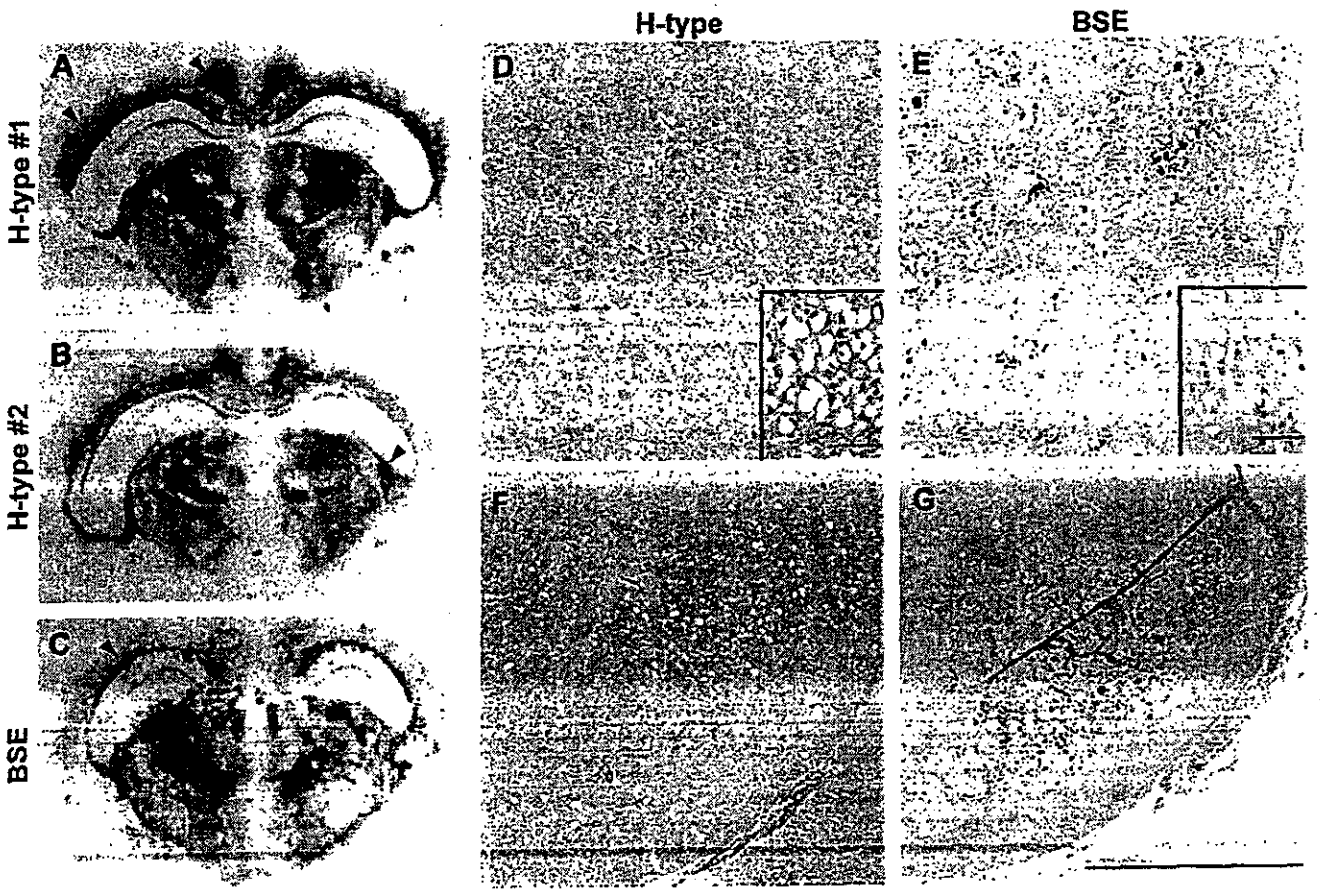


**Figure 3.** Regional Distribution of $PrP^{res}$ and Vacuolar Changes in the Brains of Bovine Transgenic Mice Infected with H-Type or BSE Agents

Histoblots of representative coronal sections of tgBov mouse brains at the levels of the hippocampus are shown. The distribution of $PrP^{res}$ deposits was similar among H-type isolates (A) (B), and different from that of cattle BSE (C) in several areas indicated by arrowheads, such as the cortex, the corpus callosum and dorsal commissure, alveus, fimbria, and stratum oriens of the hippocampus. Note that intensity of PrP deposition markedly differed between H-type and BSE agents. Illustration of how this appears by immunohistochemistry in the striatum (D) (E) and substantia nigra (F) (G), H-type–infected mice being less intensively labelled than those infected with BSE agent. By contrast, spongiosis was much more severe in H-type–infected brains. Bars: 30 μm; insert: 7 μm.

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secondary transmission, as assessed by histoblotting on brain coronal sections (Figure 3A–3B). The staining did not differ from that seen with cattle BSE in several regions, such as the striatum and several nuclei of the thalamus, including the geniculate, ventral postero-lateral and -medial as well as the brain stem (Figure 3A–3C and data not shown). However, other areas such as the cerebral cortex, the corpus callosum including the cingulum, the dorsal commissure, the alveus, and fimbria of the hippocampus were predominantly stained with H-type, whereas BSE $PrP^{res}$ was rather confined in the stratum oriens of the hippocampus (Figure 3A–3C). Moreover, the overall intensity and aspect of PrP deposition markedly differed between the two types of agents. While immunochemistry revealed various types of PrP deposits in both cases, thin diffuse PrP deposits were predominant in H-type–infected brains, whereas the most frequent type was granular in BSE-infected mouse brains. In several areas, including the striatum and the substantia nigra (Figure 3D–3G), there was a striking lack of correlation between the intensity of PrP deposition and the severity of the vacuolation. Overall, the vacuolation was much more intense in the case of H-type variant (Figures 3 and 4): areas such as the septum, hypothalamus, hippocampus, and cortex showed severe spongiosis, accompanied by a pronounced reactive glial astrocytosis based on GFAP staining (not shown), while BSE-induced vacuolation was moderate in the same areas.

## H-Type Agent Is Distinct from the Ovine TSE Isolates Transmitted so Far to tgOv Mice

We finally examined whether H-type isolates may have an ovine TSE origin. The majority of typical and atypical sheep scrapie isolates we have studied so far transmits before a year to tgOv mice ([13,14] and our unpublished data). Only a group of sheep scrapie isolates from Italy (SSit) was found to infect tgOv mice after a prolonged survival time within the range of H-type cases. Indeed three of them, SSit cases no. 5, no. 7, and no. 8, induced a typical neurodegenerative disease with a mean survival time of 698 ± 20 d (5/5 animals affected), 659 ± 31 d (7/7), and 569 ± 37 d (4/4), respectively. Case no. 5 incubation time was still longer than H-type case no. 2 on subpassage (417 ± 20 d, 6/6 animals affected). The $PrP^{res}$ molecular profile observed in the brain of SSit-diseased mice

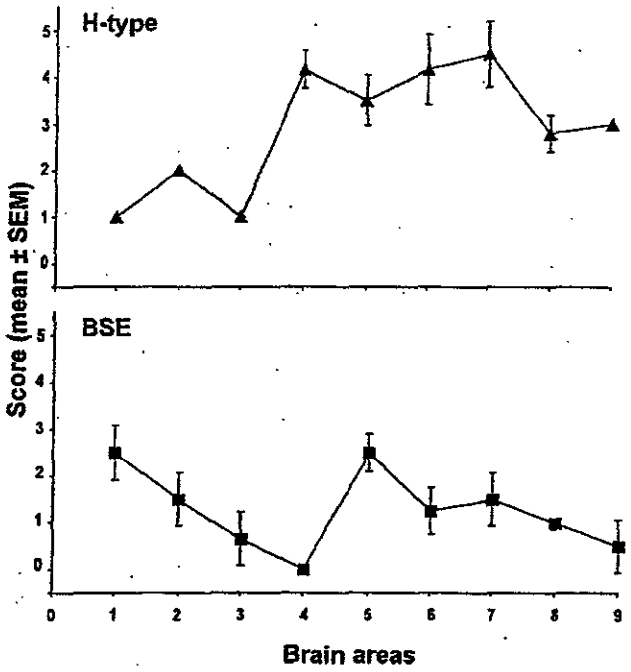


**Figure 4.** Lesion Profiles in tgBov Mice Infected with H-Type or BSE Agents

Mean scores (± SEM) reflecting the intensity of vacuolation are shown for H-type (no. 1 and no. 3, triangles) and BSE (no. 1 and no. 3, squares). The nine grey-matter areas used to construct the profile are as follows: dorsal medulla (1); cerebellar cortex (2); superior colliculus (3); hypothalamus (4); medial thalamus (5); hippocampus (6); septum (7); medial cerebral cortex at the level of the thalamus (8); and medial cerebral cortex at the level of the septum (9).

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(more than four brains analysed per isolate) was characterised by a significantly higher apparent molecular mass (difference of 0.7 ± 0.2 kDa) and a slightly higher proportion of diglycosylated $PrP^{res}$ as compared with H-type–derived $PrP^{res}$ (Figure 5A–5C). These features were conserved on secondary transmission (Figure 5A–5C). Another difference was again the pronounced accumulation of SSit-associated $PrP^{res}$ in the spleen, while this was still impaired in H-type–infected mice, even after two passages (three to five spleens tested per combination; Figures 2B and 5A).

We then compared by histoblot distribution and nature of $PrP^{res}$ deposits within the brains of tgOv mice infected with a second passage of H-type (no. 2) and SSit (no. 5) cases. Both markedly differed between the two agents as illustrated in Figure 5. Indeed, large plaques of SSit-associated $PrP^{res}$ were present in the pretectal nuclei and in related structures of the limbic system such the fornix, the alveus, fimbria, and subiculum of the hippocampus. H-type–associated $PrP^{res}$ was detected instead in the corpus callosum, cortex, and ventromedial thalamic nuclei (Figure 5D and unpublished data). The deposits seem rather thin or granular. SSit case no. 8, which gave the shortest incubation period in tgOv mice, was also inoculated by intracerebral route to tgBov mice. Of note, no disease has been observed yet in mice monitored up to 600 d after infection. In conclusion, these data suggest that the H-type agent is unrelated to the ovine TSE isolates transmitted so far to our transgenic lines.

## Discussion

In this study we show that cattle brain samples positive for abnormal PrP with a distinct molecular pattern, called H-type, consistently produces a fatal, TSE-like disease upon inoculation to both bovine and ovine PrP transgenic mice. These results, corroborating the recent transmission to wild-type mice [18], formally establish that such cases involve an authentic TSE infectious agent. Importantly, we provide detailed evidence that this newly recognised agent differs from epizootic BSE agent derived from cattle or other species.

Both molecular and biological criteria support the conclusion that H-type and BSE agents are distinct prion strains. First, the incubation periods upon transmission to mice expressing either bovine or ovine PrP produced different patterns. Thus, while primary transmission to tgOv mice led to longer survival times for both agents, the increase relative to tgBov mice was significantly less for H-type than for BSE-type agents (Figure 1). Second, the molecular profiles of the $PrP^{res}$ fragments detected in the brain of diseased mice were clearly distinguishable in either line. Strikingly, differences observed in terms of fragment size and glycoform ratio were essentially the same as in cattle brain. Third, unlike that for BSE agents, no $PrP^{res}$ signal could be seen in the spleen of H-type–infected tgOv mice, indicative of a stronger neurotropism at least in this host. Fourth, histopathological examination of tgBov mice revealed a contrasting picture. Typically, severe spongiosis and diffuse PrP deposition were present in several areas of H-type–infected brains, while the same areas of BSE-infected brains showed limited spongiosis together with marked PrP deposition. Such discrepancies are unlikely to result from unequal survival times since they were also observed on secondary passage, where the two agents had comparable incubation duration (unpublished data).

The isolation from cattle of a prion strain distinct from the one implicated in the BSE epidemics raises several concerns. One is whether H-type isolates might result from an exposure to prions of small ruminants via alimentary or environmental sources, since cattle have been shown to be susceptible to experimental infection by sheep scrapie agent [19]. In this regards, the better compatibility between ovine PrP sequence and H-type as compared to BSE was intriguing (Figure 1). However, our investigations do not support this. Among the five groups of natural isolates we have identified so far in tgOv mice ([13,14] and our unpublished data), only one group, made up mostly of SSit isolates, proposed to be of iatrogenic origin [20], showed an incubation time as prolonged as for H-type cases. However, the $PrP^{res}$ molecular profile, nature of deposits, and distribution within the brain as well as the differential accumulation in the spleen strongly distinguish H-type and SSit isolates. In addition, the latter failed so far to transmit to tgBov mice.

H-type and BSE agents might be related despite their distinguishable phenotypes. The isolation of an additional strain upon exposure of transgenic or wild-type mice to the epizootic BSE agent has been reported [21], thus questioning its strain homogeneity. Also, molecular typing studies have revealed the presence of a minor, non-BSE-type $PrP^{res}$ component in BSE- and vCJD-infected brains [22]. Hence, H-type isolates could arise from the preferential amplification in certain individuals of a subcomponent present in BSE

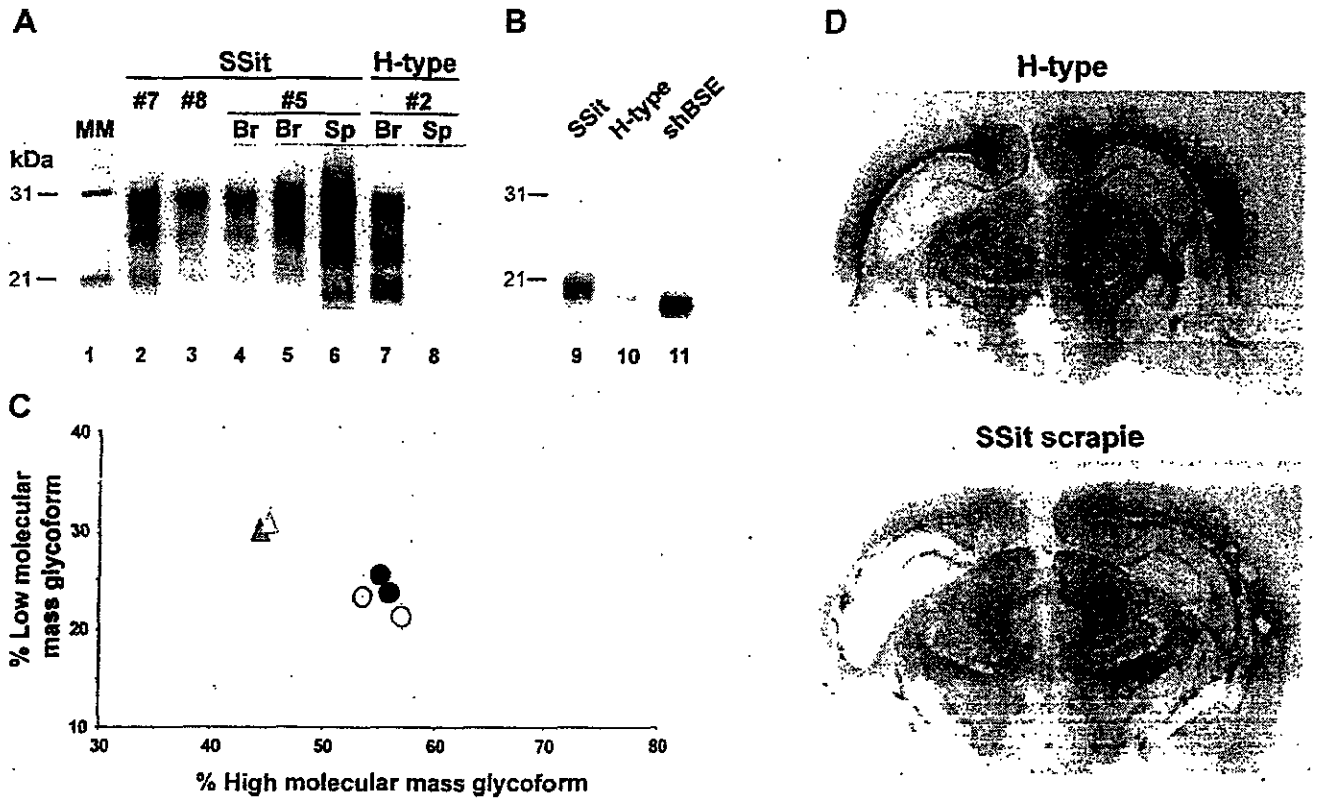


**Figure 5.** Comparison of H-Type and SSit Isolate Features upon Transmission to tgOv Mice

(A and B) Western blot analysis of $PrP^{res}$ in the brains and spleens of tgOv mice infected with H-type or SSit isolates at first (lanes 2–4) and second passage (lanes 5–11). H-type $PrP^{res}$ shows a distinct pattern in the brain (Br) compared to SSit. The apparent molecular mass of SSit $PrP^{res}$ is higher than that of H-type or sheep BSE (shBSE), as shown after PNGase treatment (B). Note also that $PrP^{res}$ is detected in the spleen (Sp) of SSit- but not of H-type-infected mice. Tissue equivalent loaded: 1.5 mg in lanes 2–4; 0.04 mg in lane 5; 0.5 mg in lanes 6–7; 2 mg in lane 8; 0.01 mg in lane 9; 0.1 mg in lanes 10–11. MM, molecular markers.

(C) Ratio of high- and low-molecular-mass $PrP^{res}$ glycoforms in the brain of tgOv mice infected with H-type or SSit isolates (data plotted as mean ± SEM). One H-type isolate (no. 2) is represented as orange triangle. SSit isolates are represented as circles (SSit no. 5, red; no. 7, brown; no. 8, yellow). Secondary transmissions are represented by unfilled symbols of the same colour. Note the stably distinct glycoform ratios between H-type and SSit agents upon serial passage.

(D) Regional distribution of $PrP^{res}$ in the brain of tgOv mice infected with H-type or SSit isolates. Histoblots of representative coronal sections of tgOv mouse brains at the levels of the hippocampus are shown. The distribution of H-type–associated $PrP^{res}$ deposits was different from that of SSit in regions such as the alveus of the hippocampus, the corpus callosum, the pretectal nuclei, the cortex, and the ventromedial thalamus. Note that the size of $PrP^{res}$ deposits markedly differed between the two types of isolates.

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infectious sources. Comparing H-type and BSE-derived variant prions identified in mice might be informative in that respect.

Alternatively, such unusual cases could reflect the existence of a natural, sporadic disease in cattle. Although it is unclear yet if such infections may lead to a clinical disease in the natural host, they seem to occur at a low frequency, which is reminiscent of the situation known for sporadic CJD in humans [23,24]. Of note, the disparities between intensity of PrP deposition and severity of vacuolation in the brains of H-type–inoculated tgBov mice have also been observed with sporadic CJD both in human or mouse infected brains [21,25]. These data, however, need to be consolidated through further investigations, including epidemiological analysis. Indeed, an implication of this latter scenario is that such bovine "atypical" cases could occur in countries free of BSE exposure. The acquisition of novel properties by an endogenous, sporadic cattle TSE agent, as occasioned on passage through an intermediary host or a physicochemical treatment such as that applied to carcass-derived products, has been invoked as one possible origin for the emergence of BSE epidemics [7]. With the isolation of such agents, we can now address this issue experimentally.

In conclusion, our findings support the view that at least two and potentially three [10] distinct prion strains may be present in cattle. The current uncertainties regarding the origin, prevalence, and potential risk for humans of a strain of TSE agent unrecognised until recently should support continued efforts to characterise it in vivo and uphold the surveillance exerted on cattle.

## Materials and Methods

**Isolates.** The H-type [9], goat BSE (CH636 case [26]), and experimental sheep BSE samples [27] were provided by the French TSE Reference Laboratory (Agence Française de Sécurité Sanitaire des Aliments [AFSSA], Lyon, France). The samples from French BSE cases and from experimental sheep BSE (ARR [$Ala^{136}Arg^{154}Arg^{171}$] genotype [28]) were provided by the Institut National de la Recherche Agronomique (INRA; Toulouse, France) and the Institute for Animal

Health (IAH) Neuropathogenesis Unit (Edinburgh, United Kingdom), respectively. The vCJD isolate was a World Health Organization (WHO) reference sample from the National Institute for Biological Standards and Control (NIBSC; Potters Bar, United Kingdom). SSit isolates were provided by the Instituto Superiore di Sanita (ISS; Rome, Italy).

**Transgenic mice and transmission assays.** The tg540 line expresses the bovine PrP allele with 6 octarepeats under the control of the cytomegalovirus (CMV) promoter on a FVB mouse line with $PrP^{0/0}$ background (Protocol S1). The tg338 line expresses the $V_{136}R_{154}Q_{171}$ allele of ovine PrP at a homozygous state, on a mouse $PrP^{0/0}$ background [29]. The transgene construct (tg3) consists in a bacterial artificial chromosome (BAC) insert of 125 kb of sheep DNA [13]. All experiments were performed according to national guidelines. Each inoculum was prepared extemporaneously in a class II microbiological cabinet using disposable equipment. Individually identified 6- to 10-wk-old mice were inoculated intracerebrally with 20 μl of a 10% (wt/vol) brain homogenate in 5% glucose. Mice were monitored daily once ill and killed in extremis.

**Analysis of $PrP^{res}$ molecular pattern.** All procedures regarding purification and detection of $PrP^{res}$ from brains and spleens of infected mice were as described [14]. ICSM18 [30] or Sha31 [31] anti-PrP antibodies were used. Enzymatic deglycosylation was performed on denatured $PrP^{res}$ with 1,000 U of recombinant PNGase (New England Biolabs, Beverly, Massachusetts, United States) for 2 h at 37 °C in 1% Nonidet P40 and the proprietary buffer as described [30]. Determination of glycoform ratio and apparent molecular mass was performed with the GeneTools software after acquisition of chemiluminescent signals with a GeneGnome digital imager (Syngene, Frederick, Maryland, United States).

**Histopathology.** For histoblot analysis [32], brains were rapidly removed from killed mice and frozen on dry ice. Thick 10-μm cryostat sections were cut, transferred onto Superfrost slides, and kept at −20 °C until use. The procedure was performed as described [14] using the 12F10 anti-PrP antibody [33]. All immunohistochemistry procedures regarding tissue processing have been described previously [34]. Samples were fixed in neutral-buffered 10% formalin (4% formaldehyde) before paraffin embedding. After deparaffinisation, 6-μm-thick tissue sections were stained with haematoxylin/eosin. Vacuolation profiles were established, following the standard method described by Fraser and Dickinson [15], by using two to three brains per isolate.

## Supporting Information

**Protocol S1.** Description of the Bovine PrP Transgenic Mice (tg540 Line)

Found at DOI: 10.1371/journal.ppat.0020112.sd001 (92 KB DOC).

### Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) accession numbers for the genes and gene products discussed in this paper are bovine PrP (NM181015) and sheep PrP (M31313).

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**Author contributions.** JLV and HL conceived and designed the experiments. VB, AB, ALD, FR, TLL, NC, GT, and JLV performed the experiments. VB, AB, and HL analyzed the data. AGB and TB contributed reagents/materials/analysis tools. VB and HL wrote the paper.

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**Competing interests.** The authors have declared that no competing interests exist.

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# 医薬品 研究報告 調査報告書

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**研究報告の概要**

○ニュージーランド食品安全局はBSE輸入規制を改訂

ニュージーランド食品安全局(NZFSA)は、BSEを取り巻く最新の科学と実際の経験を踏まえて、ウシ及びウシ加工品の輸入規制を改訂する方針である。NZFSAの規制担当者は「1996年の旧規制は、不確実性の多い時期に実施された予防的取り組みが含まれているが、その後BSEとヒトの健康へのリスクについて多くのことが判明した」と話している。

新しい規制は、科学的証拠や最近の国際的な規制に合致したものとするため、ニュージーランドにウシやウシ加工品を輸出する国のBSEのリスクステータスの分類に、国際的に認められた3カテゴリーシステムを導入する。他の改訂内容は以下の通りである。

* 独自の方法ではなく、国際的なリスク分析を導入する。
* BSEの潜在的リスクのある国からの危険部位と認められた内臓肉の輸入を禁止する。
* この規制の対象となる商品由来のごく少量のウシ由来成分を含む加工食品は例外とする。
* 輸入品の受け入れ可否と何らかの証明の必要性を決定する強固な枠組みを構築する。
* 商品の原料については年齢制限を解除し、トレーサビリティの手段を特定しない。
* 原材料、リスクのある国からの輸入を問わず、全てのゼラチンの売買を自由化する。

最近の研究では、骨由来のゼラチンの製造工程で使用される科学的処理によってBSEの感染性を充分に不活化出来ることが確認された。

新しい規制は2007年6月29日から発効するが、ゼラチンの売買自由化はそれ以前から実施される。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ニュージーランド食品安全局がウシ及びウシ加工品の輸入規制を緩和したとの報告である。 | 今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(5)

Back

**Archive Number** 20070302.0734
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**Subject** PRO/AH/EDR> BSE, bovine - New Zealand: new import rules

BSE, BOVINE - NEW ZEALAND: NEW IMPORT RULES
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Date: Wed 28 Feb 2007
From: Stuart MacDiarmid <Stuart.MacDiarmid@maf.govt.nz>
Source: NZFSA (New Zealand Food Safety Authority) official press release [edited]
<http://www.nzfsa.govt.nz/publications/media-releases/2007-02-28.htm>

NZFSA updates BSE importing requirements
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New Zealand Food Safety Authority (NZFSA) has moved to modernise the food safety importing requirements for beef and beef products in light of the new science and practical knowledge that now surrounds bovine spongiform encephalopathy (BSE).

The changes reflect recent findings from the growing body of science that more accurately identifies the risks and measures required to protect consumers from variant Creutzfeldt-Jakob disease (vCJD), the human disease that has been linked to eating beef offal containing the BSE agent.

"The old measures, in place since 1996 and adopted internationally as well as by New Zealand, reflected a precautionary approach which was taken during a time of uncertainty," explains Tim Knox, NZFSA's New Zealand standards director. "However, in the intervening years much has been learned about BSE and the risks to human health which has increased our understanding and virtually eliminated the risk of consumers contacting v[illegible]."

The new measures have undergone a comprehensive process of review and expert consideration to ensure they are consistent with scientific evidence and the emerging international standards. As a result New Zealand will move to an internationally agreed 3-category system for categorising the BSE risk status of those countries exporting beef and or beef-related products to New Zealand.

* adopt international risk assessments rather than conducting its own
* exclude offal that has been identified as BSE risk material from any country with a residual risk of BSE
* exempt processed foods that contain minimal bovine ingredients from those commodities that are covered by the measure
* adopt a consistent framework for determining the acceptability of imported products and the need for any certification
* remove age restrictions on the source of commodities and not specify measures to provide for traceability
* allow all gelatine to be traded freely, regardless of the source raw material and exporting country's BSE risk status.

Gelatine derived from bones was originally considered a risk because of fears that it could contain the BSE prion. However, recent studies have confirmed that chemical processes used in its manufacture are sufficient to inactivate any BSE infectivity that may have been present in the raw material, even under worst-case conditions. "Gelatine produced by modern

industrial processes does not pose a BSE risk to consumers, regardless of the raw material from which it is produced and the source country from which it is derived," explains Mr Knox.

The new requirements will come into effect on 29 Jun 2007 although gelatine will be freely traded before then.

Strict controls have been in place for beef products around the world since 1996 when vCJD was linked to eating beef products contaminated with offal that had come from cattle with BSE in the 1980s. Since that time, however, changes to production rules (such as not feeding ruminant material to ruminants and removing the parts of the animal from the food chain that could contain the BSE agent) have dramatically reduced the incidence of BSE.

The new requirements are not related to New Zealand's domestic animal health measures regarding BSE and will not override current animal health requirements, such as the ruminant-to-ruminant feed ban.

New Zealand has been classified as BSE-free by the World Organisation for Animal Health (OIE) and is regarded by the European Food Safety Authority as a country in which BSE is "highly unlikely" to be present.

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All information on the NZFSA website is subject to a disclaimer:
<http://www.nzfsa.govt.nz/site/disclaimer/standard.htm>.

--
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[The new rules reflect much of the same rules as other countries. - Mod.TG]

[see also:
BSE & Scrapie - New Zealand: Free 19960725.1327]

.....tg/mj/sh

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医薬品
医薬部外品　研究報告　調査報告書
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| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 9 月 29 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Guideline on validation of immunoassay for the detection of hepatitis B virus surface antigen (HBs Ag) in plasma pools.<br>London, 21 September 2006<br>EMEA/CHMP/BWP/298390/2009 | 公表国<br>米国 | |
| 販売名（企業名） | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 調査書類の評価中に見受けられた，プール血漿のヒト B 型肝炎抗原 (HBsAg) 検査におけるバリデーションの不備のある点を改善するためにこのガイドラインが作成された。HBsAg 測定法は血漿分画製剤の原料となるプール血漿内の HBsAg を検出する定性試験である。しかし，市販の HBsAg 検出キットは個人供血サンプルに対してのみバリデーションされている。したがって，血漿分画製剤用プール血漿における検査・バリデーションが必要となる。しかし，プール血漿の血清学的検査は，ウイルス感染の安全性の保証を意味するのではなく，GMP ガイドラインに対する重大な不備を検出する方法とみなされるべきである。市販キットのバリデーションとは，頑健性 (信頼性) はもちろん，特異性と検出感度 (抗原の検出限界値やカットオフ値) の決定を含む多段階プロセスである。加えて，分析法内・分析法間での変動も多数の方法を用いて行うべきである。試験方法は，準備，保管管理，試験，再試験，及び判定の手順に関する情報を含む標準作業手順書 (SOPs) で文書化されなければならない。また，弱い HBsAg 陽性コントロール (個人供血のカットオフ値の 2-3 倍) を試験ごとに組み入れるべきである。陽性サンプルは複数の異なる抗体を用いてバリデーションされた血清学的検査で陰性と実証されない限り，陽性と見なすべきである。核酸増幅検査 (NAT) は陰性を実証する方法としは使用不可能である。 | BYL-2007-0243 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| EMEA から発行された本ガイダンスは，血漿分画製剤に使用されるプール血漿の HBsAg スクリーニングの改善と標準化を目的としている。EMEA は，個人供血の試験用に開発された市販の HBsAg 検出キットをプール血漿に適応する場合，バリデーションを完全に行うよう勧告している。バリデーションされた確認法は陽性サンプルの再試験に対しても実施すべきであると考える。また，医薬品市販承認取得者と血漿マスターファイル所持者はこのガイドラインに照らし合わせてプール血漿の試験法のバリデーションを検討する必要がある。 | 本ガイドラインに記載された重要事項に対して，弊社の血漿分画製剤に使用されるプール血漿は，既存のバリデーションで適用できていることが確認されていることから，現時点では新たな対応は必要ない。 |

European Medicines Agency
*Pre-authorisation Evaluation of Medicines for Human Use*

London, 21 September 2006
EMEA/CHMP/BWP/298390/2005

**COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)**

# GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBSAG) IN PLASMA POOLS

| | |
|---|---|
| **DRAFT AGREED BY BIOLOGICS WORKING PARTY** | June 2005 |
| **ADOPTION BY CHMP FOR RELEASE FOR CONSULTATION** | September 2005 |
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| **AGREED BY BIOLOGICS WORKING PARTY** | September 2006 |
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| **DATE FOR COMING INTO EFFECT** | 1 April 2007 |

| | |
|---|---|
| **KEYWORDS** | HBsAg |

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**GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBSAG) IN PLASMA POOLS**

**TABLE OF CONTENTS**


1. SCOPE ..... 3
2. SELECTION OF THE TEST KIT(S) ..... 4
3. VALIDATION ..... 4
4. QUALITY ASSURANCE ..... 5
5. CONFIRMATION STRATEGIES ..... 6
6. IMPLEMENTATION OF THIS GUIDELINE ..... 6

## VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF HEPATITIS B VIRUS SURFACE ANTIGEN (HBsAg) IN PLASMA POOLS

### Introduction

Immunoassays for the detection of hepatitis B virus surface antigen (HBsAg) are qualitative tests for the presence of HBsAg in pooled plasma for fractionation. The validation requirements are laid down in the following documents:

- The test is considered to be a qualitative limit test for the control of impurities. Therefore, according to the "Note for guidance on validation of analytical procedures: definitions and terminology (CPMP/ICH/381/95)", published in "The rules governing medicinal products in the European Union", ICH topic Q2A, the two characteristics regarded as the most important for validation of the analytical procedure are specificity and the detection limit. However, the note for guidance adds "those validation characteristics are regarded as the most important, (...) but occasional exceptions should be dealt with on a case-by-case basis" and requires that robustness needs to be considered.
- The Ph. Eur. Monograph 01/2005:0853 "Human plasma for fractionation" requires the use of HBsAg test methods of suitable sensitivity and specificity for plasma pool testing.
- The "Note for guidance on plasma derived medicinal products" (CPMP/BWP/269/95, 3.2.2) specifies that the sensitivity of the test in relation to pool size has to be stated. The intention of the test is defined to be a safeguard against errors in testing or pooling.
- The Ph. Eur. chapter 2.7.1 "Immunochemical methods" requires the use of international reference material. Furthermore, the chapter suggests the use of commercial assay kits.
- The GMP Guide (Volume 4, Chapter 6, 6.21) as well as ISO 17025 (4.6.2) require critical reagents to be under control.

In accordance with these guidelines, the validation characteristics are described as:

- Specificity is the ability to unequivocally assess HBsAg in the presence of other components which may be expected to be present.
- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. In the context of plasma pool testing for HBsAg, the detection limit should be expressed in IU/ml with reference to the International Standard.
- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal performance.

### Validation Guidelines

### 1. SCOPE

HBsAg assay kits, for single donations purposes, marketed in Europe are CE-marked devices and are classified in Annex II A of the Directive 98/79/EC on in vitro diagnostic medical devices. The kits are therefore subject to the Common Technical Specifications (CTS, 2002/364/EC).

HBsAg assay kits are validated for use in single donation testing either through CE-marking in the EU or through non-EU regulation. The use of such assays for the testing of pooled plasma for fractionation is a change of the intended use and this application is not covered by the validation undertaken by the test kit manufacturer. The application for testing plasma pools for fractionation therefore has to be accompanied by appropriate validation.

If non-CE marked test kits are used for plasma pool testing purposes, equivalent quality to a CE-marked test kit for individual donation testing should be proven in addition to the validation for pool testing described in this guideline.

The CTS defines minimal requirements for diagnostic and analytical sensitivity for assay kits.

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Furthermore, the CTS requires that specificity should be demonstrated in a broad variety of patient samples. However, this approach to validation is not necessarily relevant for plasma pool testing purposes, because those patient samples which may give aberrant responses (e.g., those from patients with autoimmune diseases or having cross-reactive infections) will normally have been excluded by the donor selection process. In addition, non-specific interfering factors will be diluted in the plasma pool.

Plasma pool serology is not capable of detecting all contaminated single donations that may have escaped single donation screening. Patients with occult or asymptomatic Hepatitis B infection show low antigen levels that will escape detection after dilution in a manufacturing pool. Moreover, in general plasma pools may contain antibodies to HBsAg (predominantly from vaccinees), that may result in the formation of HBsAg/anti-HBs complexes which may affect the detection limit.

Plasma pool serology should therefore not be considered as a test to ensure viral safety, but as a measure to detect serious GMP failures.

This document describes methods to select and validate commercial qualitative immunoassay test kits for assessing contamination of plasma pools with HBsAg based on the above-mentioned documents.

## 2. SELECTION OF THE TEST KIT(S)

Commercial kits used for the analytical procedure are validated by the manufacturer for single donation testing only. Selection of a test kit for plasma pool testing should be based on a high analytical sensitivity.

In most cases, the manufacturers' instructions for use of reagents are adequate for the performance of the test procedure on plasma pools.

Any modification of the manufacturer's instructions should be included in the validation of use of a kit for testing plasma pools.

Evaluation criteria of the manufacturer may be adapted to plasma pool testing according to validation data relevant to plasma pools (see 3.1. Specificity and determination of a cut-off limit for pool samples, and 4. Quality assurance).

## 3. VALIDATION

### 3.1 Specificity and determination of a cut-off limit for pool samples

For commercial kits the cut-off value established by the manufacturer is a compromise between sensitivity and specificity based on results from single donation testing. Many test kit manufacturers also define a 'grey-zone' cut-off which will identify samples which give a response above background but below the cut-off. It is recommended that such samples are re-tested as if they were reactive.

On the basis of previous experience in testing plasma pools, the use of a lower cut-off for pool samples should be considered as non-specific factors present in single donations are diluted in a fractionation pool. The use of such a cut-off will increase the analytical sensitivity of assays and facilitate the detection of a single positive donation in a plasma pool. The grey-zone value recommended by the kit manufacturer may be suitable. Alternatively, a limit could be established by considering the signal distribution of negative pools, e.g. as mean response to cut-off ratios of negative pool samples + 3 standard deviations and routinely expressed as % of single donation cut-off (see 3.2.1). In no case should the pool cut-off be higher than the single donation cut-off.

For all practical applications in the context of this Guideline, if a grey-zone limit is used as the cut-off value for pool samples, this limit is used to identify initially and repeatedly reactive plasma pools (see 5., confirmation strategies).

### 3.2 Robustness

Robustness of the analytical procedure has to be evaluated, as all methods using biological and biochemical reagents may be subject of considerable batch-to-batch variation of the reagents used and may be influenced by changes in ambient conditions. Special attention has to be focussed on handling and storage of samples before testing.

#### 3.2.1 Inter-assay and intra-assay

Qualitative immunoassays primarily produce a quantitative signal that is compared to the calculated cut-off in an independent step. Reactive plasma pool samples are likely to give low signals due to high dilution in the pool. Batch-to-batch variability of the test kit reagents (including controls) may have a significant influence on results and should be under control, as is foreseen by the GMP guide (chapter 6, 6.21) and ISO 17025 (4.6.2).

Robustness of the method should be demonstrated for a panel of representative negative pool samples (e.g. routine pool samples which have tested negative by both the manufacturer and an OMCL), a low positive sample (e.g. the low positive control, see 4.3) and a freshly prepared titration series of a standard calibrated in IU in a typical plasma pool.

The study should cover:

- Inter-assay variability in 6 independent assays (including variable ambient conditions, equipment if available, preferably using more than one test kit lot if available)
- Intra-assay variability with at least 6 determinations of a low positive control in 1 run

  Intra-assay variability can be expressed as %CV (Relative Standard Deviation, RSD) of the signal of the low positive sample in relation to the cut-off of the individual assay (S/CO, sample to cut-off ratio).

#### 3.2.2 Impact of sample preparation

HBsAg may be masked by complex formation with antibodies to HBsAg (present in any European or US pool, predominantly from vaccinees) in a manner dependent on time, temperature and anti-HBs concentration. Formation of immune complexes in pooled plasma at fractionation temperature is a rather slow process (approx. 3-4 days for 50% signal loss), so efforts should be taken to minimise the time from sampling to freezing, and thawing to testing in order to avoid false negative results. For the same reason, freshly thawed samples should be used for re-testing/confirmation.

### 3.3 Detection limit

The detection limit with the pool cut-off established according to 3.1 should be determined using reference material calibrated in IU and diluted in an anti-HBs free matrix representative of a plasma pool (e.g., single plasma donation(s) or a pool of 10 single donations). It is expected that this will be considerably lower than the minimum requirement defined by the CTS.

The impact of the anti-HBs containing matrix may be assessed by data comparing the results of a titration of an HBsAg positive sample in anti-HBs positive and anti-HBs negative dilution matrix (see section 3.2.1). Where possible, this should simulate the "worst case scenario" in respect to the time that a typical pool of donations is mixed prior to sampling as well as with respect to anti-HBs concentration, temperature and agitation.

## 4. QUALITY ASSURANCE

### 4.1 Standard Operating Procedures for plasma pool testing

The test procedures must be described in detail in the form of standard operating procedures (SOPs). These should cover at least the following operations:

- Storage conditions for samples
- Preparation of samples (e.g. freezing/thawing steps, mixing)

- Description of the equipment and the test kit used
- Incubation procedures (including tolerance limits for time and temperature, e.g. according to the test kit manufacturers specifications/instrument settings)
- Detailed formulae for calculation and interpretation of results
- Validity criteria for the individual assay
- Retesting procedures
- Reference to confirmation procedures, if applicable

### 4.2 Test kit controls

The test kit manufacturer's controls should always be included in every assay to ensure correct functioning of reagents according to the manufacturers specifications. Validity criteria for modified testing conditions should be defined and documented.

### 4.3 Test kit independent controls

The positive controls in many commercial test kits are highly reactive and therefore do not reflect the low level of reactivity likely to be found in contaminated pool samples. In addition, as for all biological reagents, these controls are subject of batch-to-batch variation. Therefore it is strongly advised to include an independent low positive control (in the dynamic range of the assay, e.g. 2-3 times the single donation cut-off) in every test used for on-going data monitoring.

### 4.4 Proficiency testing

Regular participation in an appropriate proficiency testing scheme which include diluted samples with low reactivity to assess the analytical sensitivity of kits is encouraged.

## 5. CONFIRMATION STRATEGIES

A validated confirmation strategy for initially reactive results should be in place. A pool is considered negative if a fresh aliquot of the initially reactive sample gives a negative result when retested in duplicate. Repeat reactive samples have to be considered positive unless proven otherwise with a validated serological method using different antibodies (alternative assay, neutralization with neutralization reagent).

For HBsAg testing, a neutralization test should always be used on repeat reactive samples. The neutralization test has to be validated for pool plasma samples, considering the neutralization effect of antibodies already present in the pool (self-neutralization) in comparison to the neutralized sample (incubated with additional anti-HBs).

As HBsAg may be present in donors with low or undetectable nucleic acid plasma levels, NAT should not be considered as a confirmation assay as a negative NAT results does not invalidate a positive serological result. On the other hand, positive NAT results do confirm the serological detection of contaminations.

## 6. IMPLEMENTATION OF THIS GUIDELINE

This guideline has been developed to respond to inadequacies in the validation of plasma pool testing for HBsAg observed during evaluation of dossiers. Marketing Authorisation Holders and Plasma Master File Holders should review the validation of their pool testing methods in the light of this guidance. If the key aspects described in the guideline have already been covered by existing validation, no further validation is needed. If this is not the case, pool testing should be validated in accordance with this guideline and reported in the next annual update of the documentation on the plasma starting material.

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**研究報告の概要**

○個別核酸増幅検査は、全ての輸血によるB型肝炎ウイルス伝播を防止することにはならない
2004年10月、神奈川県赤十字血液センターは、輸血後HBV感染疑いの報告を受けた。受血者は急性骨髄芽球性白血病の51歳の男性で、2004年1月に骨髄移植を受けた。受血者に輸血された血液を供血した33人の供血者の凍結検体を回収した。供血当時の検査では50プールNAT陰性だったにも関わらず、凍結検体がHBV個別NAT陽性となった54歳日本人男性の供血者を特定した。供血者は、これ以前の11年間に78回の血小板成分供血を行っていた。
この供血者の以前の供血に関する遡及調査にあたって、異なる病院で発生した未解決の輸血後B型肝炎疑いの3症例を調査した。患者は3名とも複数回の血小板輸血を受けており、製剤のうちそれぞれ1製剤はこの供血者由来だった。後方視的検査では、この3回の供血血液の凍結検体はHBV個別NAT陰性だった。しかし、供血者と患者4名のHBVの塩基配列を比較したところ、全てジェノタイプCで相同性は99.8%だった。
供血者の過去の供血の40の凍結検体について個別NAT を行うことができた。受血者の面接調査とHBVマーカー検査により、過去のHBV感染が更に2例特定された。これらの患者のうち臨床的に明らかな輸血後B型肝炎の既往のある者はいなかった。回収された検体のうち幾つかは陰性、幾つかは陽性だった。この供血者におけるHBV DNAの量は50コピー/mL未満から200コピー/mLの間で増減していた。
本症例は、供血前に個別NATを行ったとしても、全てのオカルトHBVキャリアを排除できないことを示している。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 供血者の個別NATは陰性だったが、塩基配列から輸血による感染が確認された輸血後B型肝炎の症例が発生したとの報告である。 | HBV感染に関する新たな知見等について今後も情報の収集に努める。日本赤十字社では、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。また、NATの精度向上についても評価・検討している。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

自発報告:1-04000222、1-03000103、1-0400072、1-04000269、1-04000271、1-04000278、1-04000348

# LETTERS TO THE EDITOR

## Individual nucleic amplification technology does not prevent all hepatitis B virus transmission by blood transfusion

We report a case of posttransfusion hepatitis B for which the donor's pretransfusion 50-minipool hepatitis B virus (HBV) nucleic amplification technology (NAT) test result was negative, but retrospective testing of the same sample by individual-donation (ID) NAT was positive. We were able to test 40 frozen samples from prior collections from this donor using HBV ID-NAT and to test current blood samples from pertinent recipients for evidence of prior HBV infection. We believe that the results of this study show that predonation testing by HBV ID-NAT would not have prevented all HBV transmissions by this donor.

In October 2004, the Kanagawa Red Cross Blood Center received a report of a suspected case of posttransfusion hepatitis B. The patient was a 51-year-old man with acute myeloblastic leukemia who had received a marrow transplant in January 2004. We retrieved frozen samples from 33 donors whose blood was transfused to this patient. We identified one donor, a 54-year-old Japanese man, whose frozen sample from the implicated donation tested positive by HBV ID-NAT, although it had tested negative by 50-sample minipool NAT at the time of donation. This person had donated 78 apheresis platelet (PLT) units during the prior 11 years.

To initiate a lookback study of prior donations by this donor, we began with a review of three unresolved cases of suspected posttransfusion hepatitis B from three different hospitals. All three patients had received multiple PLT transfusions, including 1 unit each from the implicated donor. Retrospective testing of frozen blood samples from these three donations was negative by HBV ID-NAT. DNA sequences of the HBV of this donor and the four patients who had developed posttransfusion hepatitis B, however, were analyzed. All were genotype C and had 99.8 percent homology. We were able to test 40 frozen samples from this donor's prior donations (Table 1). Interviews with recipients and the results of HBV marker testing of these samples identified two additional cases of prior HBV infection. None of these patients had a history of clinically apparent post-transfusion hepatitis B. Some of these retrieved samples tested negative and some tested positive by HBV ID-NAT (Table 1). The quantitative HBV DNA in this donor blood fluctuated between less than 50 copies per/mL to 200 copies per/mL.

The results of this study raise serious and difficult issues for Japanese blood banks. Although the existence of HBV carriers has been known, it has been anticipated that most HBV-infective donations would be detected by minipool NAT.[1] Our case study shows that even ID-NAT does not detect all occult HBV carriers. Unfortunately, HBV is endemic in Japan and certain other countries in Asia. Our present strategy to eliminate hepatitis B surface antigen (HBsAg)-negative and /HBV-infective donors is based on the method of Mosley and colleagues,[2] namely, excluding antibody to hepatitis B core (anti-HBc)-positive donations, unless HBsAg antibody is unequivocally positive. Our anti-HBc assay is hemmagglutination inhibition with a twofold step dilution (cutoff level, $>2^5$). This assay eliminates 1.6 percent of our donors, but less than 2 percent of donors excluded by this approach tested positive by HBV ID-NAT. We cannot implement more and more sensitive anti-HBc assay, such as enzyme immu-

**TABLE 1. Results of HBV ID-NAT testing of frozen blood samples of implicated donor and responses of recipients of his blood**

| Number of blood donations | Date of donation | Component | ID-NAT | Recipient |
|---|---|---|---|---|
| 1 | 04/12/04 | PC | + | Not infected |
| 2 | 03/25/04 | PC | + | |
| 3 | 03/10/04 | PC | + | Suspected SC |
| 4 | 02/23/04 | NT | + | |
| 5 | 02/06/04 | PC | + | |
| 6* | 01/19/04 | PC | + | Acute hepatitis |
| 7 | 12/29/03 | PC | | SC |
| 8 | 12/04/03 | PC | | |
| 9 | 10/22/03 | PC | + | Suspected SC |
| 10 | 09/22/03 | PC | | Infected |
| 11 | 07/28/03 | NT | | |
| 12 | 07/14/03 | PC | | Not infected |
| 13 | 06/09/03 | PC | + | |
| 14 | 01/13/03 | PC | | |
| 15 | 12/12/02 | NT | | |
| 16 | 08/28/02 | Whole blood | | |
| 17 | 06/15/02 | PC | | Acute hepatitis |
| 18 | 05/22/02 | PC | | |
| 19 | 04/22/02 | PC | | Acute hepatitis |
| 20 | 03/14/02 | NT | | |
| 21 | 01/21/02 | PC | | Not infected |
| 22 | 12/28/01 | NT | | |
| 23 | 11/21/01 | PC | | |
| 24 | 10/29/01 | NT | | |
| 25 | 10/09/01 | PC | | |
| 26 | 09/14/01 | PC | | |
| 27 | 08/25/01 | PC | | |
| 28 | 07/31/01 | NT | | |
| 29 | 07/17/01 | PC | | |
| 30 | 06/26/01 | PC | | |
| 31 | 06/07/01 | PC | | Not infected |
| 32 | 05/22/01 | PC | | |
| 33 | 08/16/99 | NT | + | |
| 34 | 07/29/99 | PC | | |
| 35 | 07/31/98 | NT | | |
| 36 | 10/16/97 | FFP | | |
| 37 | 08/17/97 | PC | | |
| 38 | 07/05/97 | PC | | |
| 39 | 06/08/97 | NT | | |
| 40 | 05/17/97 | PC | | Acute hepatitis |

* The propositus case.
† Infection report from hospital.
PC = PLT concentrate; SC = seroconversion; NT = not transfused.

noassay, because loss of 20 percent of our donors is not feasible for a national blood program. Other measures will be necessary to further decrease posttransfusion HBV infection. Some of the options include prophylaxis using HBV immunization or high-titer hepatitis B immunoglobulin before beginning chemotherapy or monitoring transfusion recipients for HBV DNA by ID-NAT monthly to detect HBV infection in time to administer anti-HBV drugs, such as lamivudine or adefovir dipivoxil.

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## Are weak D red blood cells really immunogenic?

In a recent letter to the editor, Kumpel[1] questioned the immunogenicity of weak D red blood cells (RBCs). Kumpel described five case reports that were published during the prior 6 years that offered neither evidence that single units of weak D can immunize in the absence of other alloantigens nor evidence of clinical significance. That letter was followed by replies from the authors who had published the five case reports. Of the 28 references cited in the discussion, only one, listed in both the first and the last of the exchanged communications, described any clinical trial of antigenicity.[2] That research was performed under circumstances remarkably like those pertaining today; that is, there had been a redefinition of what was then called D$^u$, just as DNA typing is now redefining weak D. The circumstances of 50 years ago are just as pertinent today. In 1956, the blood bank (now Department of Transfusion Medicine) at the National Institutes of Health (NIH) Clinical Center was part of the Division of Biologics Standards that held the licensing authority for reagents now vested in the Food and Drug Administration. We began to study the licensed technology of the day by applying it in the actual care of patients at the clinical center hospital. In the practice of many blood bankers then, D$^u$ still had the specter of an antigen in its original 1946 definition; that is, RBCs not agglutinated by complete (saline) anti-Rh but agglutinated by incomplete (high protein) anti-Rh.[3] At NIH, we had progressed to calling D$^u$ only those RBCs not agglutinated by incomplete reagents but agglutinated in the indirect antiglobulin (Coombs) test.

Our trial of D$^u$ took several years and it has never been repeated. We transfused 68 units of D$^u$ RBCs to 49 D– patients who had no evidence of any prior immunization. The survival of the transfused RBCs was followed by the Ashby (differential agglutination) technique. Three-fourths of the patients were studied for longer than 5 months. The study has been challenged because 15 of the patients were receiving therapy that might have interfered with an antibody response, but none of the D– recipients of D$^u$ made anti-D. Some were certainly presented simultaneously with other alloantigens, with one patient making anti-K and another anti-E.[2]

The argument continued for 30 more years until a half-solution came out of an informal meeting of parties in Raritan, New Jersey, with Peter Agre (he received his Nobel Prize for something else). The successful idea that resulted was to simply castrate D$^u$ by changing its name to weak D.[4]

Now with molecular typing, weak D can be sorted into dozens of alleles, but Kumpel[1] points out that none of the five case reports of immunization by weak D describe any mortality or morbidity. Despite earlier cautions,[5] there are proposals that all weak D types found in blood donors be sorted into their multiple possibilities. Even with availability of the valuable new DNA typing, that redefinition of D$^u$ should not be translated into burdensome limita-

# 医薬品 研究報告 調査報告書

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|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 米国 |

**研究報告の概要**

○血液の安全性に危険を及ぼす可能性から供血停止となった供血者における特定のウイルス感染症の有病率

【背景】健康歴の問診により、感染症伝播のリスクを高めると考えられる供血者が特定される。本試験は、現在この問診が血液の安全性に及ぼす影響を、感染症マーカーを示すことにより評価する。

【試験デザインおよび方法】健康歴の問診によって供血延期となった供血者を、4つの米国赤十字血液センターで募集し、血液感染症の血清マーカーについて血液検体を検査した。

【結果】登録された供血停止者497名のうち、29名は"11歳以降の黄疸、肝臓疾患、肝炎"の発症のために供血停止となった。このうち1名はC型肝炎ウイルス(HCV)抗体陽性かつB型肝炎コア抗体(HBc抗体)陽性、2名はHBc抗体陽性、1名はHCV抗体陽性だった(両マーカーともp<0.05)。

"過去の肝炎検査で陽性であった"ため供血停止となった37名のうち、1名はB型肝炎表面抗原陽性かつHBc抗体陽性、3名はHBc抗体陽性であった(両マーカーともp<0.05)。

"これまでに(1度でも)非合法、非処方薬を投与するための注射針を使用したことがある"ために供血停止となった14名のうち、1名はHCV抗体陽性、ヒトT-リンパ球向性ウイルス1型抗体陽性かつHBc抗体陽性であり、1名はHCV抗体陽性かつHBc抗体陽性、2名はHCV抗体陽性であった(いずれのマーカーもp<0.05)。

【結論】ウイルス肝炎リスクおよび静注薬物使用歴に関する標準的な供血者用問診にて供血停止となった供血者は、供血停止とならなかった供血者よりも肝炎マーカー陽性率が高い場合が多かった。その他のマーカーおよび質問について有意な知見は認めなかった。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| ウイルス肝炎リスクおよび静注薬物使用歴に関する問診にて不適となった供血者は、供血可能となった者よりも肝炎マーカー陽性率が高い場合が多かったとの報告である。 | 日本赤十字社は、輸血感染症対策として、慢性B型肝炎・C型肝炎キャリア、非合法薬物の注射については 献血不可としている。今後も引き続き情報の収集に努める。 |



9

# Prevalence of selected viral infections among blood donors deferred for potential risk to blood safety

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**BACKGROUND:** Health history questions identify blood donors believed to pose a higher risk of transmission of infectious diseases. This study assesses the current impact of some of these questions on blood safety as reflected by infectious disease markers.

**STUDY DESIGN AND METHODS:** Donors who were deferred from donating blood due to health history question(s) were recruited at four different regions of the American Red Cross Blood Services. A blood sample was tested for serologic markers of blood-borne infections as performed for accepted blood donors.

**RESULTS:** Of 497 deferred donors enrolled, 29 donors were deferred for having had "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), 1 of whom had hepatitis C virus antibodies (anti-HCV) and hepatitis B core antigen antibodies (anti-HBc), 2 had anti-HBc, and 1 had anti-HCV ($p < 0.05$ for both markers). Among 37 donors deferred for having "ever tested positive for hepatitis" (Question 4), 1 had hepatitis B surface antigen and anti-HBc and 3 had anti-HBc ($p < 0.05$ for both markers). Of 14 donors deferred for "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12), 1 had anti-HCV, human T-lymphotropic virus-I antibodies and anti-HBc, 1 had anti-HCV and anti-HBc, and 2 had anti-HCV ($p < 0.05$ for all three markers).

**CONCLUSIONS:** Blood donors deferred for standard blood donor questions regarding risk of viral hepatitis as well as those with a history of intravenous drug use were more likely to have higher hepatitis marker rates than those who were not deferred. No significant findings were identified for other markers or questions.

The safety of blood collected for transfusion is ensured through appropriate procedures for donor recruitment, education, health history, and testing of donated blood units.[1] Safe donors are encouraged to donate their blood whereas at-risk donors are encouraged to self-defer from blood donation. At blood collection sites, presenting donors are informed of known or newly identified risks of blood-borne infections to help their decision making regarding donation. Presenting donors are further interviewed for history of potential exposure to transmissible diseases that are caused by blood-borne infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-lymphotropic virus (HTLV). Donors who are believed to be at an increased risk for those infections are deferred from making a donation.

During the blood donor interview, donors are screened through medical examination and a questionnaire for health history. The examination and questions

**ABBREVIATIONS:** ARC = American Red Cross; IVDU = intravenous drug use; UDHQ = universal donor history questionnaire.

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are related to either the safety of donors or their potential risk of exposure to infectious diseases.[2] For example, date of last donation, date of birth, hemoglobin level, blood pressure, pulse, and weight are mainly for the safety of blood donors, whereas histories of, or exposure to, HIV, hepatitis, and other blood-borne, sexually transmitted, or certain endemic infections are for the safety of blood recipients. Donors in the first category are deferred from donating blood since the donation process may harm their health. Donors in the latter category are deferred from donating blood to prevent blood recipients from exposure to potentially higher risk of infectious diseases. Such donors may be harboring an infectious agent and potentially be able to transmit that agent to recipients through the donated blood. For blood-borne infections for which no testing is available, blood donor interview and subsequent deferral is believed to represent an important safeguard against transfusion transmission of such infections. Even for blood-borne infections for which there is routine testing, donor interview and deferral are considered extra layers of assurance, because blood donor infectious disease testing, while having excellent sensitivity, is not without false-negative results, particularly for very recently acquired infections.

The expanding list of health history questions related to potential exposure to blood-borne infections has had a significant impact on the blood collection process. Not only have such questions resulted in a large number of blood donors being deferred each year by blood centers in the United States but also many of the temporarily deferred donors do not return to donate blood.[2-7] Few studies have been conducted to evaluate the interview process or the majority of the screening questions. With the changing epidemiology of blood-borne infections among blood donors and the improved testing methods for donated blood, an assessment of the current impact of the interview process and the screening questions has become more relevant.

An earlier study of American Red Cross (ARC) blood donors examined prevalence of hepatitis B surface antigen (HBsAg) or antibodies to HIV (anti-HIV), HCV (anti-HCV), or HTLV (anti-HTLV) for different groups of donors who were temporarily deferred in 2000 to 2001 and later returned to donate blood in 2000 to 2003.[8] The results were compared with either first-time or repeat donors in 2000 to 2003, while controlling for differences in sex, age, and year of donation. Of donors temporarily deferred in 2000 to 2001 who had had no donation or deferral during the previous 2 years, only 22.08 percent subsequently returned to donate blood in 2000 to 2003. Donations from returning donors who had been deferred for potential infectious disease risk did not show a higher prevalence for any of the viral markers when those with no donation or deferral during the previous 2 years were compared with first-time donations and those with prior donation were compared with repeat donations. The study concluded that blood donors temporarily deferred in 2000 to 2001 for potential risk of viral infection who later returned to donate blood did not appear to pose a higher risk compared to first-time or repeat donors. The study suggested that the effectiveness of some of the currently used deferral questions in reducing viral risks warranted further study. The previous study, however, only examined interview questions that result in temporary deferrals. In addition, a limitation of this study was that temporarily deferred donors who subsequently returned to donate blood could be different from those who had been deferred but did not return.

To further assess the impact of the donor health history questionnaire and particularly certain interview questions that result in indefinite deferral, a study was designed to recruit deferred donors and to test a sample of their blood for prevalence of blood-borne infections.

## MATERIALS AND METHODS

### Study population

The study targeted blood donors who presented to ARC Blood Services and were deferred from donating blood due to answering "yes" to health history question(s) at four different centers of the ARC Blood Services during the following time periods: 2003 to 2005 for two centers, 2004 to 2005 for a third center, and 1999 to 2004 for a fourth center. The first two centers were located in New England, the third center in the Southeast, and the fourth center in the Upper Midwest. In the fourth center, only donors who were deferred for hepatitis, including hepatitis A and hepatitis with infectious mononucleosis, or for intravenous drug use (IVDU; Questions 3, 4, and 12) were targeted for recruitment. A yes answer to any of these questions resulted in indefinite deferral. In the other three centers, however, donors who were deferred for all health history questions related to recipient safety (see Appendix), indefinite or temporary, were targeted for recruitment. The study was approved by the ARC Institutional Review Board.

### Recruitment

In the first three centers, deferred donors were recruited through a variety of means, including personal invitations at blood drives, mailing of invitation letters, phone calls, or a combination of mailing and phone calls. For recruitment at blood drives, donation databases were used to target larger blood drives, and research staff attended these targeted drives. For recruitment through mailing with or without follow-up phone call, donation databases were searched for all deferred donors who had been deferred for the targeted questions during the targeted period and were residing in areas surrounding certain

fixed collection sites. Donors deferred at drives where personal recruitment occurred were excluded from the mailing and/or phone approach. Letters were then sent to the selected deferred donors to inform them of the study and to ask them to contact research staff to arrange for a blood draw. Approximately 2 weeks after the mailing, follow-up phone calls were made to some of those who had not responded to the mailing. For the fourth center, phone call was the only method used for recruitment and donors were called multiple times, if necessary, to make contact.

### Data collection

In addition to data on deferral, demographic data on deferred donors including sex, age, and previous donor presentations were obtained from a research database of donors and donations (ARCNET database). The ARCNET Data Center maintains data of blood donors and donations as well as donor deferrals, excluding name or other information that would allow identification of individual donors, for all Blood Services Regions of the ARC since 1995.[9] A blood sample was collected from each enrolled deferred donor and tested for serologic markers of blood-borne infections as for successful blood donors. During the period covered by this study, all donations were tested as described previously for anti-HIV, anti-HCV, anti-HTLV, HBsAg, and antibodies to HBV core antigen (anti-HBc).[9] During this period, nucleic acid testing (NAT) was also performed on blood donations for detecting HIV RNA and HCV RNA.[10,11] NAT of deferred donors, however, was not included in this study. All enrolled deferred donors were tested with the same serologic screening and confirmatory tests used for nondeferred donors. The results of confirmatory testing are reported for HBsAg, anti-HCV, anti-HIV, and anti-HTLV. Because there is no confirmatory test for the hepatitis B core antibodies, the results of screening testing are reported. Positive test rates were compared to those for first-time donors from the same region because first-time donors have higher marker rates than repeat donors and first-time donors account for most of donors deferred for potential infection risks.

### Statistical analysis

Statistical analyses were carried out with computer software (SAS, SAS Institute, Cary, NC).[12] Comparisons were performed with a t test for means, with a chi-square test for proportions, or with probability determination based on Poisson distribution where appropriate. Analysis of factors that may be associated with increased or decreased risk of blood-borne infections among enrolled deferred donors was further performed with Poisson regression with SAS.[13] For the analysis, sex, and age of deferred donors as well as participating region (center) were included in the model. All reported p values are two-sided. A p value of less than 0.05 or a 95 percent confidence interval (CI) of an odds ratio (OR) that does not include 1 indicates that a difference is considered to be significant.

## RESULTS

### Comparison of enrolled versus nonenrolled deferred donors

In total, 497 deferred donors were enrolled for this study, with a mean enrollment rate of 13 percent. Enrollment rates varied with different recruiting methods and among the four participating blood services regions. Invitation at blood drives gave higher enrollment rates but lower overall yield due to limitation of available research staff to attend blood drives. With three of the four centers combined, recruitment at blood drives obtained an enrollment rate of 49.2 percent, whereas mailing alone gave only an enrollment rate of 5.0 percent. Mailing with a follow-up phone call was able to enroll 11.7 percent of the targeted deferred donors. At the fourth center where repeated phone calls were the only recruitment method used, 28.7 percent of targeted deferred donors were enrolled.

Compared with nonenrolled deferred donors, those who were enrolled were less likely to be male (42% among enrolled vs. 51% among nonenrolled, $p < 0.05$), more likely to be older (40 years old on average among enrolled vs. 34 years among nonenrolled, $p < 0.05$), likely to have made more prior donations (a mean of 4.1 times among enrolled vs. 1.5 times among nonenrolled, $p < 0.05$), and also likely to have been deferred more often (0.8 times on average among enrolled vs. 0.5 times among nonenrolled, $p < 0.05$). Compared to first-time donors who donated to the same regions during the period of the study but were not deferred, those who were enrolled were less likely to be male (42% vs. 49%, $p < 0.05$) and more likely to be older (40 years old vs. 31 years old, $p < 0.05$). Table 1 shows the comparison of enrolled donors versus nonenrolled donors who had been deferred for several specified reasons. Owing to the small number of enrollees, comparisons for other deferral questions are not shown. For donors deferred for Questions 12 (intravenous drug use [IVDU]) and 19 (blood exposures), enrolled donors had fewer male donors compared to nonenrolled donors, whereas for other questions, differences in sex composition between enrolled and nonenrolled groups are not significant. For donors deferred for Question 3 (liver disease), 4 (positive hepatitis test), 19, and 37 (HIV-1 Group O exposure), enrolled donors tended to be older than nonenrolled donors. For donors deferred for Questions 3, 4, and 19, enrolled donors appeared to have presented more times for donation than nonenrolled donors. Enrolled donors who were deferred for Question 19 also appeared to have had more prior deferrals than nonenrolled donors.

### Testing results for infectious disease markers among enrolled donors

Confirmed positive tests for anti-HCV, anti-HIV, HBsAg, and anti-HTLV and repeat-reactive tests for anti-HBc are shown in Table 2 for enrolled donors who had been deferred for Questions 3, 4, 12, 17, 19, or 37. None of the enrolled donors was confirmed positive for anti-HIV. No confirmed positive for any of the above markers was identified among enrolled donors who had been deferred for other questions. The testing results were compared to those for first-time donors who donated to the same regions during the period of the study but were not deferred. Of the 29 donors who were deferred for having had "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), 1 had anti-HCV and anti-HBc, 2 had anti-HBc, and 1 had anti-HCV ($p < 0.05$ for anti-HCV and anti-HBc). Thirty-seven donors were deferred for having "ever tested positive for hepatitis" (Question 4), 1 of whom had HBsAg and anti-HBc and 3 had anti-HBc ($p < 0.05$ for HBsAg and anti-HBc). Among the 14 donors deferred for "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12), 1 had anti-HCV, anti-HTLV-I, and anti-HBc; 1 had anti-HCV and anti-HBc; and 2 had anti-HCV ($p < 0.05$ for all). Sixty-nine donors were deferred for travel to malarial areas (Question 17), 1 of whom had anti-HBc (not significant [NS]). Of the 127 donors deferred for a history in the past 12 months of "tattooing, ear/body piercing, acupuncture, accidental needlestick, coming into contact with someone else's blood, or taking (snorting) cocaine or any other street drug through one's nose" (Question 19), 1 was anti-HBc repeat-reactive (NS). Among 18 donors deferred for possible exposure in certain African countries (Question 37), 1 had anti-HBc (NS).

**TABLE 1. Comparison of enrolled versus nonenrolled deferred donors**

| Deferral question | Enrolled | Nonenrolled | p Value |
|---|---|---|---|
| Percentage who were male | | | |
| 3 (liver disease) | 55.2 | 53.0 | 0.822 |
| 4 (hepatitis positive) | 45.9 | 53.2 | 0.400 |
| 12 (IVDU) | 50.0 | 79.2 | 0.017* |
| 17 (malaria) | 55.1 | 52.6 | 0.808 |
| 19 (blood exposure) | 29.1 | 42.3 | 0.004* |
| 37 (HIV in Africa) | 22.2 | 36.0 | 0.250 |
| Mean (SD) age (years) | | | |
| 3 (liver disease) | 48.1 (15.3) | 39.8 (14.0) | 0.002* |
| 4 (hepatitis positive) | 46.0 (13.2) | 41.1 (13.9) | 0.040* |
| 12 (IVDU) | 36.1 (10.2) | 36.9 (12.1) | 0.809 |
| 17 (malaria) | 35.5 (15.0) | 35.7 (15.2) | 0.947 |
| 19 (blood exposure) | 35.1 (11.9) | 28.5 (11.3) | <0.001* |
| 37 (HIV in Africa) | 38.0 (12.6) | 32.0 (10.3) | 0.025* |
| Mean (SD) number of prior donations | | | |
| 3 (liver disease) | 1.6 (3.2) | 0.6 (1.4) | <0.001* |
| 4 (hepatitis positive) | 1.8 (3.1) | 0.9 (2.6) | 0.047* |
| 12 (IVDU) | 0.3 (0.5) | 0.4 (1.5) | 0.785 |
| 17 (malaria) | 5.2 (8.2) | 4.1 (8.4) | 0.484 |
| 19 (blood exposure) | 2.6 (3.4) | 1.5 (3.2) | <0.001* |
| 37 (HIV in Africa) | 0.4 (0.7) | 0.9 (2.4) | 0.442 |
| Mean (SD) number of prior deferrals | | | |
| 3 (liver disease) | 0.6 (0.7) | 0.4 (0.7) | 0.068 |
| 4 (hepatitis positive) | 0.7 (1.0) | 0.5 (1.0) | 0.303 |
| 12 (IVDU) | 0.4 (0.6) | 0.2 (0.5) | 0.223 |
| 17 (malaria) | 0.7 (0.6) | 0.6 (0.6) | 0.346 |
| 19 (blood exposure) | 0.8 (0.9) | 0.5 (0.6) | <0.001* |
| 37 (HIV in Africa) | 0.3 (0.5) | 0.3 (0.5) | 0.776 |

* p Values of significance.

### Poisson regression analysis of marker-positive rates

Because the enrolled deferred donor group differs from first-time donors in composition of sex and age, comparison between the enrolled group and first-time donors needs to take into account such differences. Poisson regression was used to compare the marker-positive rates in the enrolled deferred donors with those among first-time donors while incorporating into the model these two factors as well as participating blood center. Owing to the relationship of Questions 3 and 4 and their small numbers, results for the two questions were combined for the Poisson regression analysis. Further, owing to the small numbers, testing results for different markers were combined. If a donor tested positive for the presence of at least one of anti-HIV, anti-HCV, anti-HTLV, or HBsAg, or anti-HBc repeat-reactive, the testing result was defined as "marker-positive." The results are shown in Table 3. Essentially, donors deferred for Questions 3, 4, and 12 showed higher positive rates of these viral markers than first-time

**TABLE 2. Confirmatory results for infectious disease markers among enrolled deferred donors (repeatedly reactive *results for anti-HBc*)**

| Deferral question | Number deferred | Anti-HBc | Anti-HCV | HBsAg/Anti-HBc | Anti-HCV/Anti-HBc | Anti-HCV/Anti-HBc/Anti-HTLV | Count by individual test: Anti-HBc | HBsAg | Anti-HCV | Anti-HIV | Anti-HTLV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 (liver disease) | 29 | 2 | 1 | 0 | 1 | 0 | 3* | 0 | 2 | 0 | 0 |
| 4 (hepatitis positive) | 37 | 3 | 0 | 1 | 0 | 0 | 4* | 1* | 0 | 0 | 0 |
| 12 (IVDU) | 14 | 0 | 2 | 0 | 1 | 1 | 2* | 0 | 4* | 0 | 1* |
| 17 (malaria) | 69 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 19 (blood exposure) | 127 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 37 (HIV in Africa) | 18 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

* p Value < 0.05 based on Poisson distribution

**TABLE 3. Poisson regression analysis of marker-positive rates**

| Deferral question | OR (95% CI) | p Value |
|---|---|---|
| 3 (liver disease) and 4 (hepatitis) | 4.68 (2.34-9.37) | <0.01 |
| 12 (IVDU) | 18.13 (6.80-48.31) | <0.01 |
| 17 (malaria) | 0.84 (0.12-5.97) | 0.86 |
| 19 (blood exposure) | 0.53 (0.07-3.75) | 0.52 |
| 37 (HIV-1 group O risk) | 3.40 (0.48-24.18) | 0.22 |

donors but donors deferred for Questions 17, 19, or 37 did not show higher viral marker rates when potential differences in sex and age composition as well as intercenter variations were taken into account. Similar results were obtained for Questions 3, 4, and 12 when anti-HBc was excluded in the comparison.

## DISCUSSION

Our previous study showed that blood donors temporarily deferred for potential risk of viral infection who later returned to donate blood did not appear to pose a higher risk compared to first-time or repeat donors.[5] In contrast, results from the present study show that donors indefinitely deferred for "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), "ever tested positive for hepatitis" (Question 4), or "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12) were more likely to have higher hepatitis marker rates than those who were not deferred. These questions result in indefinite deferrals. Owing to low response rate among deferred donors and potential recruitment bias of deferred donors, who may not reflect potential donors with that deferral factor in the general population, caution should be used, however, when generalizing these data to the larger population of potential blood donors.

Among other specific reasons for deferral, Question 19, namely, "In the past 12 months, have you had a tattoo, ear/body piercing, acupuncture, accidental needlestick, come into contact with someone else's blood, or taken (snorted) cocaine or any other street drug through your nose," warrants further discussion. The question caused more than 4 percent of all of the temporary deferrals and has been studied in both the previous analysis[5] and this investigation. Results from our previous study did not show an increased risk of viral infections by HIV, HBV, HCV, or HTLV among donors who were deferred for this question and subsequently returned to donate blood.[5] The prevalence (/100,000) among those with no prior donation or deferral history was 126.6 for anti-HCV, 14.1 for anti-HIV, 70.3 for HBsAg, 14.1 for anti-HTLV, or 211.0 for any of these markers, compared to 287.7, 11.4, 74.3, 10.9, or 380.5 for first-time volunteer donors. None of the 6036 donors who were deferred for this question and had had a prior donation were positive for the presence of any of the viral markers, compared to a prevalence (/100,000) of 6.3, 1.3, 2.1, 0.3, or 9.9 for those markers among repeat volunteer donors. Comparison through Poisson distribution showed that the differences were not significant ($p > 0.05$ for all comparisons).[5] Results from the present study show that, except for one donor who was anti-HBc repeat-reactive, none of the 127 enrolled donors deferred for this question tested positive for the presence of any of anti-HIV, anti-HCV, anti-HTLV, or HBsAg. Although the small sample size makes it impossible to draw a conclusion that donors deferred for these reasons do not pose a higher risk than first-time blood donors, the results do suggest the likelihood of such risks. For anti-HCV, calculation of probabilities indicates that at the current prevalence level of anti-HCV among first-time blood donors the likelihood of having one or more anti-HCV–positive samples among 127 individuals exceeds 20 percent. The likelihood of having one or more anti-HCV–positive samples would increase to more than 50 percent if the prevalence rate of anti-HCV should double that among first-time donors. In other words, the likelihood of having twice as high a prevalence rate of anti-HCV among donors deferred for Question 19 compared to first-time donors should be less than 50 percent. Analysis of the anti-HBc result does not suggest a higher prevalence rate of the marker among donors deferred for Question 19.

In addition, our previous study showed that Question 17 (travel outside of the United States, for malaria infection risk) does not appear to have any merit of serving as a possible surrogate for potentially increased exposure to blood-borne or sexually transmitted infections while traveling abroad.[5] Results from this study are consistent with the earlier findings. None of the 69 enrolled donors in this study who were deferred for this question tested positive for the presence of anti-HIV, anti-HCV, anti-HTLV, or HBsAg; one was repeat-reactive for anti-HBc, which rate was not different from that among first-time donors. Neither this nor the previous study was able to assess the effectiveness of this question in reduction of malaria risk for which it was introduced.

Annually, Questions 17 and 19 result in a total of 60,000 to 70,000 deferrals for the ARC Blood Services. Our previous study[5] showed that only 24.5 percent of the donors deferred for Question 19 and 39.0 percent of the donors deferred for Question 17 returned to donate blood after their deferral. Among donors who presented the first time and were deferred for Question 19 or 17, only 18.0 or 22.7 percent, respectively, returned to donate their blood. Deferral of those donors not only results in loss of their donations when they are deferred but more importantly, a majority of those donors are lost permanently. Many safe blood donors could be kept in the blood donor pool if these two questions could be modified. In the newly

implemented universal donor history questionnaire (UDHQ), Question 19 has been divided into several individual questions.[14] A study is under way with the ARCNET database to assess the potential impact of changes associated with UDHQ, including the changes with Question 19. Along with the implementation of UDHQ, ARC Blood Services regions are allowed to collect blood from donors with tattoos applied by state regulated parlors, which could help reduce donor deferral for tattooing (accounting for most of Question 19 deferrals during the study period) while maintaining the safety of the blood supply, as suggested by an earlier study in one US blood center.[15] For Question 17, a test for malaria may be able to identify those who are truly a risk to recipients, which could allow changes be made to the question so that many safe blood donors would be able to donate.[16]

Certain lessons have been learned from the study. This study demonstrates the difficulties of recruiting deferred donors into research studies. The most successful method for recruiting deferred donors in our study was to approach deferred donors at time of deferral. The intermediate step of collection staff forwarding deferred donors to research staff complicated this process. A possible improvement to the study and thereby to the enrollment rate would be to incorporate the study into blood center operations so that collection staff can consent and collect deferred donors. This would allow recruitment at every drive, increasing the number of eligible deferred donors approached and removing the intermediate referral step.[17] The difficulty in recruiting deferred donors also shows that deferred donors are unwilling to return, which further supports our previous findings of low return rates for deferred donors.[8]

Caution needs to be exercised in extrapolating data from this study to the entire population of deferred donors or the general population for the deferral reasons included in this study. Although sex and age as well as intercenter variation have been taken into account when comparison was performed between enrolled deferred donors and first-time donors, there could be other differences between enrolled and nonenrolled deferred donors. It is likely, however, that temporarily deferred donors who consented to the present investigation do not completely overlap with temporarily deferred donors who returned to donate blood and were targeted in the previous study.[8] For example, temporarily deferred donors who returned to donate blood could be overrepresented by more committed donors compared to those who did not return. Therefore, results from the present investigation, in combination with results from the previous analysis of returned donors, shed more light on the effectiveness of the current donor selection and deferral process as well as on the utility of specific deferral questions.

In summary, donors deferred for a history of liver disease, positive test for hepatitis, or IVDU were more likely to have higher prevalence for the hepatitis markers under study. Results from this study support the original basis for introducing the questions. Unfortunately the study was too small to detect any HIV-positive donors, and we cannot make any conclusions regarding this agent. Results from this study, however, do not suggest a higher risk of hepatitis B or C infections among donors deferred for "tattooing, ear/body piercing, acupuncture, accidental needlestick, coming into contact with someone else's blood, or taking (snorting) cocaine or any other street drug through one's nose." Similarly, this study did not show an increased risk of hepatitis among donors deferred for travel to malarial areas. The study was unable to assess, however, whether these questions have any impact on other potential blood-borne pathogens that are not currently being screened for during blood collection. Because a majority of deferred donors are unlikely to return for donation following their deferral, many safe blood donors could be saved and retained if the two questions are improved. Analysis of changes in deferral and marker-positive rate associated with UDHQ implementation may be able to show whether the changes will have made a difference.

Larger studies of deferred donors may provide additional important information about the effectiveness of blood donor health history interviews. Such larger studies will almost certainly need to be performed by routine health history interviewers at a significant number of blood centers to provide sufficient statistical power to prove that any of the current donor questions are unnecessary.

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## APPENDIX

Health history questions targeted:

3. Since the age of 11, have you had yellow jaundice, liver disease, or hepatitis?
4. Have you ever tested positive for hepatitis?
5. In the past 12 months, have you been in close contact with anyone having yellow jaundice or hepatitis, or have you received hepatitis B immune globulin (HBIG)?
7. Have you ever taken clotting factor concentrates for a bleeding problem, such as hemophilia?
12. Have you ever used a needle, even once, to take any illegal or nonprescription drug?
13. Have you taken money or drugs in exchange for sex since 1977?
14. Are you a male who has had sex, even once, with another male since 1977?
17. In the past 12 months, have you traveled outside of the U.S. except Canada, Australia, New Zealand, Japan, or Western Europe, including the British Isles?
18. In the past 12 months, have you received a blood transfusion or an organ or tissue transplant?
19. In the past 12 months, have you had a tattoo, ear/body piercing, acupuncture, accidental needlestick, come into contact with someone else's blood, or taken (snorted) cocaine or any other street drug through your nose?
21. In the past 12 months, have you had or been treated for syphilis or gonorrhea or tested positive for syphilis?
22. In the past 12 months, have you had sex, even once, with anyone who has ever used a needle for illegal or nonprescription drugs?
23. In the past 12 months, have you had sex, even once, with anyone who has taken money or drugs in exchange for sex since 1977?
24. In the past 12 months, have you given money or drugs to anyone to have sex with you?
25. In the past 12 months, have you had sex, even once, with anyone who has taken clotting factor concentrates?
26. In the past 12 months, have you had sex, even once, with anyone who has had AIDS or tested positive for the AIDS virus?
27. Are you a female who, in the past 12 months, has had sex with a male who has had sex, even once, with another male since 1977?
35. Do you have AIDS or have you ever tested positive for the AIDS virus?
37. (a) Were you born in, or have you lived in, Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger, or Nigeria, since 1977? (b) Since 1977, have you received a blood transfusion or medical treatment with a blood product in any of these countries? (c) Have you had sex with anyone who, since 1977, was born in or lived in any of these countries?

# 医薬品 研究報告 調査報告書

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**研究報告の概要**

○血液の安全性に危険を及ぼす可能性から供血停止となった供血者における特定のウイルス感染症の有病率

【背景】健康歴の問診により、感染症伝播のリスクを高めると考えられる供血者が特定される。本試験は、現在この問診が血液の安全性に及ぼす影響を、感染症マーカーを示すことにより評価する。

【試験デザインおよび方法】健康歴の問診によって供血延期となった供血者を、4つの米国赤十字血液センターで募集し、血液感染症の血清マーカーについて血液検体を検査した。

【結果】登録された供血停止者497名のうち、29名は"11歳以降の黄疸、肝臓疾患、肝炎"の発症のために供血停止となった。このうち1名はC型肝炎ウイルス(HCV)抗体陽性かつB型肝炎コア抗体(HBc抗体)陽性、2名はHBc抗体陽性、1名はHCV抗体陽性だった(両マーカーともp<0.05)。

"過去の肝炎検査で陽性であった"ため供血停止となった37名のうち、1名はB型肝炎表面抗原陽性かつHBc抗体陽性、3名はHBc抗体陽性であった(両マーカーともp<0.05)。

"これまでに(1度でも)非合法、非処方薬を投与するための注射針を使用したことがある"ために供血停止となった14名のうち、1名はHCV抗体陽性、ヒトT-リンパ球向性ウイルス1型抗体陽性かつHBc抗体陽性であり、1名はHCV抗体陽性かつHBc抗体陽性、2名はHCV抗体陽性であった(いずれのマーカーもp<0.05)。

【結論】ウイルス肝炎リスクおよび静注薬物使用歴に関する標準的な供血者用問診にて供血停止となった供血者は、供血停止とならなかった供血者よりも肝炎マーカー陽性率が高い場合が多かった。その他のマーカーおよび質問について有意な知見は認めなかった。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ウイルス肝炎リスクおよび静注薬物使用歴に関する問診にて不適となった供血者は、供血可能となった者よりも肝炎マーカー陽性率が高い場合が多かったとの報告である。 | 日本赤十字社は、輸血感染症対策として、慢性B型肝炎・C型肝炎キャリア、非合法薬物の注射については 献血不可としている。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

# Prevalence of selected viral infections among blood donors deferred for potential risk to blood safety

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**BACKGROUND:** Health history questions identify blood donors believed to pose a higher risk of transmission of infectious diseases. This study assesses the current impact of some of these questions on blood safety as reflected by infectious disease markers.

**STUDY DESIGN AND METHODS:** Donors who were deferred from donating blood due to health history question(s) were recruited at four different regions of the American Red Cross Blood Services. A blood sample was tested for serologic markers of blood-borne infections as performed for accepted blood donors.

**RESULTS:** Of 497 deferred donors enrolled, 29 donors were deferred for having had "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), 1 of whom had hepatitis C virus antibodies (anti-HCV) and hepatitis B core antigen antibodies (anti-HBc), 2 had anti-HBc, and 1 had anti-HCV ($p < 0.05$ for both markers). Among 37 donors deferred for having "ever tested positive for hepatitis" (Question 4), 1 had hepatitis B surface antigen and anti-HBc and 3 had anti-HBc ($p < 0.05$ for both markers). Of 14 donors deferred for "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12), 1 had anti-HCV, human T-lymphotropic virus-I antibodies and anti-HBc, 1 had anti-HCV and anti-HBc, and 2 had anti-HCV ($p < 0.05$ for all three markers).

**CONCLUSIONS:** Blood donors deferred for standard blood donor questions regarding risk of viral hepatitis as well as those with a history of intravenous drug use were more likely to have higher hepatitis marker rates than those who were not deferred. No significant findings were identified for other markers or questions.

The safety of blood collected for transfusion is ensured through appropriate procedures for donor recruitment, education, health history, and testing of donated blood units.[1] Safe donors are encouraged to donate their blood whereas at-risk donors are encouraged to self-defer from blood donation. At blood collection sites, presenting donors are informed of known or newly identified risks of blood-borne infections to help their decision making regarding donation. Presenting donors are further interviewed for history of potential exposure to transmissible diseases that are caused by blood-borne infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and human T-lymphotropic virus (HTLV). Donors who are believed to be at an increased risk for those infections are deferred from making a donation.

During the blood donor interview, donors are screened through medical examination and a questionnaire for health history. The examination and questions

**ABBREVIATIONS:** ARC = American Red Cross; IVDU = intravenous drug use; UDHQ = universal donor history questionnaire.

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are related to either the safety of donors or their potential risk of exposure to infectious diseases.[2] For example, date of last donation, date of birth, hemoglobin level, blood pressure, pulse, and weight are mainly for the safety of blood donors, whereas histories of, or exposure to, HIV, hepatitis, and other blood-borne, sexually transmitted, or certain endemic infections are for the safety of blood recipients. Donors in the first category are deferred from donating blood since the donation process may harm their health. Donors in the latter category are deferred from donating blood to prevent blood recipients from exposure to potentially higher risk of infectious diseases. Such donors may be harboring an infectious agent and potentially be able to transmit that agent to recipients through the donated blood. For blood-borne infections for which no testing is available, blood donor interview and subsequent deferral is believed to represent an important safeguard against transfusion transmission of such infections. Even for blood-borne infections for which there is routine testing, donor interview and deferral are considered extra layers of assurance, because blood donor infectious disease testing, while having excellent sensitivity, is not without false-negative results, particularly for very recently acquired infections.

The expanding list of health history questions related to potential exposure to blood-borne infections has had a significant impact on the blood collection process. Not only have such questions resulted in a large number of blood donors being deferred each year by blood centers in the United States but also many of the temporarily deferred donors do not return to donate blood.[2-7] Few studies have been conducted to evaluate the interview process or the majority of the screening questions. With the changing epidemiology of blood-borne infections among blood donors and the improved testing methods for donated blood, an assessment of the current impact of the interview process and the screening questions has become more relevant.

An earlier study of American Red Cross (ARC) blood donors examined prevalence of hepatitis B surface antigen (HBsAg) or antibodies to HIV (anti-HIV), HCV (anti-HCV), or HTLV (anti-HTLV) for different groups of donors who were temporarily deferred in 2000 to 2001 and later returned to donate blood in 2000 to 2003.[8] The results were compared with either first-time or repeat donors in 2000 to 2003, while controlling for differences in sex, age, and year of donation. Of donors temporarily deferred in 2000 to 2001 who had had no donation or deferral during the previous 2 years, only 22.08 percent subsequently returned to donate blood in 2000 to 2003. Donations from returning donors who had been deferred for potential infectious disease risk did not show a higher prevalence for any of the viral markers when those with no donation or deferral during the previous 2 years were compared with first-time donations and those with prior donation were compared with repeat donations. The study concluded that blood donors temporarily deferred in 2000 to 2001 for potential risk of viral infection who later returned to donate blood did not appear to pose a higher risk compared to first-time or repeat donors. The study suggested that the effectiveness of some of the currently used deferral questions in reducing viral risks warranted further study. The previous study, however, only examined interview questions that result in temporary deferrals. In addition, a limitation of this study was that temporarily deferred donors who subsequently returned to donate blood could be different from those who had been deferred but did not return.

To further assess the impact of the donor health history questionnaire and particularly certain interview questions that result in indefinite deferral, a study was designed to recruit deferred donors and to test a sample of their blood for prevalence of blood-borne infections.

## MATERIALS AND METHODS

### Study population

The study targeted blood donors who presented to ARC Blood Services and were deferred from donating blood due to answering "yes" to health history question(s) at four different centers of the ARC Blood Services during the following time periods: 2003 to 2005 for two centers, 2004 to 2005 for a third center, and 1999 to 2004 for a fourth center. The first two centers were located in New England, the third center in the Southeast, and the fourth center in the Upper Midwest. In the fourth center, only donors who were deferred for hepatitis, including hepatitis A and hepatitis with infectious mononucleosis, or for intravenous drug use (IVDU; Questions 3, 4, and 12) were targeted for recruitment. A yes answer to any of these questions resulted in indefinite deferral. In the other three centers, however, donors who were deferred for all health history questions related to recipient safety (see Appendix), indefinite or temporary, were targeted for recruitment. The study was approved by the ARC Institutional Review Board.

### Recruitment

In the first three centers, deferred donors were recruited through a variety of means, including personal invitations at blood drives, mailing of invitation letters, phone calls, or a combination of mailing and phone calls. For recruitment at blood drives, donation databases were used to target larger blood drives, and research staff attended these targeted drives. For recruitment through mailing with or without follow-up phone call, donation databases were searched for all deferred donors who had been deferred for the targeted questions during the targeted period and were residing in areas surrounding certain

fixed collection sites. Donors deferred at drives where personal recruitment occurred were excluded from the mailing and/or phone approach. Letters were then sent to the selected deferred donors to inform them of the study and to ask them to contact research staff to arrange for a blood draw. Approximately 2 weeks after the mailing, follow-up phone calls were made to some of those who had not responded to the mailing. For the fourth center, phone call was the only method used for recruitment and donors were called multiple times, if necessary, to make contact.

### Data collection

In addition to data on deferral, demographic data on deferred donors including sex, age, and previous donor presentations were obtained from a research database of donors and donations (ARCNET database). The ARCNET Data Center maintains data of blood donors and donations as well as donor deferrals, excluding name or other information that would allow identification of individual donors, for all Blood Services Regions of the ARC since 1995.[9] A blood sample was collected from each enrolled deferred donor and tested for serologic markers of blood-borne infections as for successful blood donors. During the period covered by this study, all donations were tested as described previously for anti-HIV, anti-HCV, anti-HTLV, HBsAg, and antibodies to HBV core antigen (anti-HBc).[9] During this period, nucleic acid testing (NAT) was also performed on blood donations for detecting HIV RNA and HCV RNA.[10,11] NAT of deferred donors, however, was not included in this study. All enrolled deferred donors were tested with the same serologic screening and confirmatory tests used for nondeferred donors. The results of confirmatory testing are reported for HBsAg, anti-HCV, anti-HIV, and anti-HTLV. Because there is no confirmatory test for the hepatitis B core antibodies, the results of screening testing are reported. Positive test rates were compared to those for first-time donors from the same region because first-time donors have higher marker rates than repeat donors and first-time donors account for most of donors deferred for potential infection risks.

### Statistical analysis

Statistical analyses were carried out with computer software (SAS, SAS Institute, Cary, NC).[12] Comparisons were performed with a t test for means, with a chi-square test for proportions, or with probability determination based on Poisson distribution where appropriate. Analysis of factors that may be associated with increased or decreased risk of blood-borne infections among enrolled deferred donors was further performed with Poisson regression with SAS.[13] For the analysis, sex, and age of deferred donors as well as participating region (center) were included in the model. All reported p values are two-sided. A p value of less than 0.05 or a 95 percent confidence interval (CI) of an odds ratio (OR) that does not include 1 indicates that a difference is considered to be significant.

## RESULTS

### Comparison of enrolled versus nonenrolled deferred donors

In total, 497 deferred donors were enrolled for this study, with a mean enrollment rate of 13 percent. Enrollment rates varied with different recruiting methods and among the four participating blood services regions. Invitation at blood drives gave higher enrollment rates but lower overall yield due to limitation of available research staff to attend blood drives. With three of the four centers combined, recruitment at blood drives obtained an enrollment rate of 49.2 percent, whereas mailing alone gave only an enrollment rate of 5.0 percent. Mailing with a follow-up phone call was able to enroll 11.7 percent of the targeted deferred donors. At the fourth center where repeated phone calls were the only recruitment method used, 28.7 percent of targeted deferred donors were enrolled.

Compared with nonenrolled deferred donors, those who were enrolled were less likely to be male (42% among enrolled vs. 51% among nonenrolled, $p < 0.05$), more likely to be older (40 years old on average among enrolled vs. 34 years among nonenrolled, $p < 0.05$), likely to have made more prior donations (a mean of 4.1 times among enrolled vs. 1.5 times among nonenrolled, $p < 0.05$), and also likely to have been deferred more often (0.8 times on average among enrolled vs. 0.5 times among nonenrolled, $p < 0.05$). Compared to first-time donors who donated to the same regions during the period of the study but were not deferred, those who were enrolled were less likely to be male (42% vs. 49%, $p < 0.05$) and more likely to be older (40 years old vs. 31 years old, $p < 0.05$). Table 1 shows the comparison of enrolled donors versus nonenrolled donors who had been deferred for several specified reasons. Owing to the small number of enrollees, comparisons for other deferral questions are not shown. For donors deferred for Questions 12 (intravenous drug use [IVDU]) and 19 (blood exposures), enrolled donors had fewer male donors compared to nonenrolled donors, whereas for other questions, differences in sex composition between enrolled and nonenrolled groups are not significant. For donors deferred for Question 3 (liver disease), 4 (positive hepatitis test), 19, and 37 (HIV-1 Group O exposure), enrolled donors tended to be older than nonenrolled donors. For donors deferred for Questions 3, 4, and 19, enrolled donors appeared to have presented more times for donation than nonenrolled donors. Enrolled donors who were deferred for Question 19 also appeared to have had more prior deferrals than nonenrolled donors.

### Testing results for infectious disease markers among enrolled donors

Confirmed positive tests for anti-HCV, anti-HIV, HBsAg, and anti-HTLV and repeat-reactive tests for anti-HBc are shown in Table 2 for enrolled donors who had been deferred for Questions 3, 4, 12, 17, 19, or 37. None of the enrolled donors was confirmed positive for anti-HIV. No confirmed positive for any of the above markers was identified among enrolled donors who had been deferred for other questions. The testing results were compared to those for first-time donors who donated to the same regions during the period of the study but were not deferred. Of the 29 donors who were deferred for having had "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), 1 had anti-HCV and anti-HBc, 2 had anti-HBc, and 1 had anti-HCV ($p < 0.05$ for anti-HCV and anti-HBc). Thirty-seven donors were deferred for having "ever tested positive for hepatitis" (Question 4), 1 of whom had HBsAg and anti-HBc and 3 had anti-HBc ($p < 0.05$ for HBsAg and anti-HBc). Among the 14 donors deferred for "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12), 1 had anti-HCV, anti-HTLV-I, and anti-HBc; 1 had anti-HCV and anti-HBc; and 2 had anti-HCV ($p < 0.05$ for all). Sixty-nine donors were deferred for travel to malarial areas (Question 17), 1 of whom had anti-HBc (not significant [NS]). Of the 127 donors deferred for a history in the past 12 months of "tattooing, ear/body piercing, acupuncture, accidental needlestick, coming into contact with someone else's blood, or taking (snorting) cocaine or any other street drug through one's nose" (Question 19), 1 was anti-HBc repeat-reactive (NS). Among 18 donors deferred for possible exposure in certain African countries (Question 37), 1 had anti-HBc (NS).

**TABLE 1. Comparison of enrolled versus nonenrolled deferred donors**

| Deferral question | Enrolled | Nonenrolled | p Value |
|---|---|---|---|
| Percentage who were male | | | |
| 3 (liver disease) | 55.2 | 53.0 | 0.822 |
| 4 (hepatitis positive) | 45.9 | 53.2 | 0.400 |
| 12 (IVDU) | 50.0 | 79.2 | 0.017* |
| 17 (malaria) | 55.1 | 52.6 | 0.808 |
| 19 (blood exposure) | 29.1 | 42.3 | 0.004* |
| 37 (HIV in Africa) | 22.2 | 36.0 | 0.250 |
| Mean (SD) age (years) | | | |
| 3 (liver disease) | 48.1 (15.3) | 39.8 (14.0) | 0.002* |
| 4 (hepatitis positive) | 46.0 (13.2) | 41.1 (13.9) | 0.040* |
| 12 (IVDU) | 36.1 (10.2) | 36.9 (12.1) | 0.809 |
| 17 (malaria) | 35.5 (15.0) | 35.7 (15.2) | 0.947 |
| 19 (blood exposure) | 35.1 (11.9) | 28.5 (11.3) | <0.001* |
| 37 (HIV in Africa) | 38.0 (12.6) | 32.0 (10.3) | 0.025* |
| Mean (SD) number of prior donations | | | |
| 3 (liver disease) | 1.6 (3.2) | 0.6 (1.4) | <0.001* |
| 4 (hepatitis positive) | 1.8 (3.1) | 0.9 (2.6) | 0.047* |
| 12 (IVDU) | 0.3 (0.5) | 0.4 (1.5) | 0.785 |
| 17 (malaria) | 5.2 (8.2) | 4.1 (8.4) | 0.484 |
| 19 (blood exposure) | 2.6 (3.4) | 1.5 (3.2) | <0.001* |
| 37 (HIV in Africa) | 0.4 (0.7) | 0.9 (2.4) | 0.442 |
| Mean (SD) number of prior deferrals | | | |
| 3 (liver disease) | 0.6 (0.7) | 0.4 (0.7) | 0.068 |
| 4 (hepatitis positive) | 0.7 (1.0) | 0.5 (1.0) | 0.303 |
| 12 (IVDU) | 0.4 (0.6) | 0.2 (0.5) | 0.223 |
| 17 (malaria) | 0.7 (0.6) | 0.6 (0.6) | 0.346 |
| 19 (blood exposure) | 0.8 (0.9) | 0.5 (0.6) | <0.001* |
| 37 (HIV in Africa) | 0.3 (0.5) | 0.3 (0.5) | 0.776 |

* p Values of significance.

### Poisson regression analysis of marker-positive rates

Because the enrolled deferred donor group differs from first-time donors in composition of sex and age, comparison between the enrolled group and first-time donors needs to take into account such differences. Poisson regression was used to compare the marker-positive rates in the enrolled deferred donors with those among first-time donors while incorporating into the model these two factors as well as participating blood center. Owing to the relationship of Questions 3 and 4 and their small numbers, results for the two questions were combined for the Poisson regression analysis. Further, owing to the small numbers, testing results for different markers were combined. If a donor tested positive for the presence of at least one of anti-HIV, anti-HCV, anti-HTLV, or HBsAg, or anti-HBc repeat-reactive, the testing result was defined as "marker-positive." The results are shown in Table 3. Essentially, donors deferred for Questions 3, 4, and 12 showed higher positive rates of these viral markers than first-time

**TABLE 2. Confirmatory results for infectious disease markers among enrolled deferred donors (repeatedly reactive results for anti-HBc)**

| Deferral question | Number deferred | Anti-HBc | Anti-HCV | HBsAg/Anti-HBc | Anti-HCV/Anti-HBc | Anti-HCV/Anti-HBc/Anti-HTLV | Count by individual test: Anti-HBc | HBsAg | Anti-HCV | Anti-HIV | Anti-HTLV |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 (liver disease) | 29 | 2 | 1 | 0 | 1 | 0 | 3* | 0 | 2 | 0 | 0 |
| 4 (hepatitis positive) | 37 | 3 | 0 | 1 | 0 | 0 | 4* | 1* | 0 | 0 | 0 |
| 12 (IVDU) | 14 | 0 | 2 | 0 | 1 | 1 | 2* | 0 | 4* | 0 | 1* |
| 17 (malaria) | 69 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 19 (blood exposure) | 127 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| 37 (HIV in Africa) | 18 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

* p Value < 0.05 based on Poisson distribution

**TABLE 3. Poisson regression analysis of marker-positive rates**

| Deferral question | OR (95% CI) | p Value |
|---|---|---|
| 3 (liver disease) and 4 (hepatitis) | 4.68 (2.34-9.37) | <0.01 |
| 12 (IVDU) | 18.13 (6.80-48.31) | <0.01 |
| 17 (malaria) | 0.84 (0.12-5.97) | 0.86 |
| 19 (blood exposure) | 0.53 (0.07-3.75) | 0.52 |
| 37 (HIV-1 group O risk) | 3.40 (0.48-24.18) | 0.22 |

donors but donors deferred for Questions 17, 19, or 37 did not show higher viral marker rates when potential differences in sex and age composition as well as intercenter variations were taken into account. Similar results were obtained for Questions 3, 4, and 12 when anti-HBc was excluded in the comparison.

## DISCUSSION

Our previous study showed that blood donors temporarily deferred for potential risk of viral infection who later returned to donate blood did not appear to pose a higher risk compared to first-time or repeat donors.[5] In contrast, results from the present study show that donors indefinitely deferred for "yellow jaundice, liver disease, or hepatitis since the age of 11" (Question 3), "ever tested positive for hepatitis" (Question 4), or "having ever used a needle, even once, to take any illegal or nonprescription drug" (Question 12) were more likely to have higher hepatitis marker rates than those who were not deferred. These questions result in indefinite deferrals. Owing to low response rate among deferred donors and potential recruitment bias of deferred donors, who may not reflect potential donors with that deferral factor in the general population, caution should be used, however, when generalizing these data to the larger population of potential blood donors.

Among other specific reasons for deferral, Question 19, namely, "In the past 12 months, have you had a tattoo, ear/body piercing, acupuncture, accidental needlestick, come into contact with someone else's blood, or taken (snorted) cocaine or any other street drug through your nose," warrants further discussion. The question caused more than 4 percent of all of the temporary deferrals and has been studied in both the previous analysis[5] and this investigation. Results from our previous study did not show an increased risk of viral infections by HIV, HBV, HCV, or HTLV among donors who were deferred for this question and subsequently returned to donate blood.[5] The prevalence (/100,000) among those with no prior donation or deferral history was 126.6 for anti-HCV, 14.1 for anti-HIV, 70.3 for HBsAg, 14.1 for anti-HTLV, or 211.0 for any of these markers, compared to 287.7, 11.4, 74.3, 10.9, or 380.5 for first-time volunteer donors. None of the 6036 donors who were deferred for this question and had had a prior donation were positive for the presence of any of the viral markers, compared to a prevalence (/100,000) of 6.3, 1.3, 2.1, 0.3, or 9.9 for those markers among repeat volunteer donors. Comparison through Poisson distribution showed that the differences were not significant ($p > 0.05$ for all comparisons).[5] Results from the present study show that, except for one donor who was anti-HBc repeat-reactive, none of the 127 enrolled donors deferred for this question tested positive for the presence of any of anti-HIV, anti-HCV, anti-HTLV, or HBsAg. Although the small sample size makes it impossible to draw a conclusion that donors deferred for these reasons do not pose a higher risk than first-time blood donors, the results do suggest the likelihood of such risks. For anti-HCV, calculation of probabilities indicates that at the current prevalence level of anti-HCV among first-time blood donors the likelihood of having one or more anti-HCV–positive samples among 127 individuals exceeds 20 percent. The likelihood of having one or more anti-HCV–positive samples would increase to more than 50 percent if the prevalence rate of anti-HCV should double that among first-time donors. In other words, the likelihood of having twice as high a prevalence rate of anti-HCV among donors deferred for Question 19 compared to first-time donors should be less than 50 percent. Analysis of the anti-HBc result does not suggest a higher prevalence rate of the marker among donors deferred for Question 19.

In addition, our previous study showed that Question 17 (travel outside of the United States, for malaria infection risk) does not appear to have any merit of serving as a possible surrogate for potentially increased exposure to blood-borne or sexually transmitted infections while traveling abroad.[6] Results from this study are consistent with the earlier findings. None of the 69 enrolled donors in this study who were deferred for this question tested positive for the presence of anti-HIV, anti-HCV, anti-HTLV, or HBsAg; one was repeat-reactive for anti-HBc, which rate was not different from that among first-time donors. Neither this nor the previous study was able to assess the effectiveness of this question in reduction of malaria risk for which it was introduced.

Annually, Questions 17 and 19 result in a total of 60,000 to 70,000 deferrals for the ARC Blood Services. Our previous study[5] showed that only 24.5 percent of the donors deferred for Question 19 and 39.0 percent of the donors deferred for Question 17 returned to donate blood after their deferral. Among donors who presented the first time and were deferred for Question 19 or 17, only 18.0 or 22.7 percent, respectively, returned to donate their blood. Deferral of those donors not only results in loss of their donations when they are deferred but more importantly, a majority of those donors are lost permanently. Many safe blood donors could be kept in the blood donor pool if these two questions could be modified. In the newly

implemented universal donor history questionnaire (UDHQ), Question 19 has been divided into several individual questions.[14] A study is under way with the ARCNET database to assess the potential impact of changes associated with UDHQ, including the changes with Question 19. Along with the implementation of UDHQ, ARC Blood Services regions are allowed to collect blood from donors with tattoos applied by state regulated parlors, which could help reduce donor deferral for tattooing (accounting for most of Question 19 deferrals during the study period) while maintaining the safety of the blood supply, as suggested by an earlier study in one US blood center.[15] For Question 17, a test for malaria may be able to identify those who are truly a risk to recipients, which could allow changes be made to the question so that many safe blood donors would be able to donate.[16]

Certain lessons have been learned from the study. This study demonstrates the difficulties of recruiting deferred donors into research studies. The most successful method for recruiting deferred donors in our study was to approach deferred donors at time of deferral. The intermediate step of collection staff forwarding deferred donors to research staff complicated this process. A possible improvement to the study and thereby to the enrollment rate would be to incorporate the study into blood center operations so that collection staff can consent and collect deferred donors. This would allow recruitment at every drive, increasing the number of eligible deferred donors approached and removing the intermediate referral step.[17] The difficulty in recruiting deferred donors also shows that deferred donors are unwilling to return, which further supports our previous findings of low return rates for deferred donors.[8]

Caution needs to be exercised in extrapolating data from this study to the entire population of deferred donors or the general population for the deferral reasons included in this study. Although sex and age as well as intercenter variation have been taken into account when comparison was performed between enrolled deferred donors and first-time donors, there could be other differences between enrolled and nonenrolled deferred donors. It is likely, however, that temporarily deferred donors who consented to the present investigation do not completely overlap with temporarily deferred donors who returned to donate blood and were targeted in the previous study.[8] For example, temporarily deferred donors who returned to donate blood could be overrepresented by more committed donors compared to those who did not return. Therefore, results from the present investigation, in combination with results from the previous analysis of returned donors, shed more light on the effectiveness of the current donor selection and deferral process as well as on the utility of specific deferral questions.

In summary, donors deferred for a history of liver disease, positive test for hepatitis, or IVDU were more likely to have higher prevalence for the hepatitis markers under study. Results from this study support the original basis for introducing the questions. Unfortunately the study was too small to detect any HIV-positive donors, and we cannot make any conclusions regarding this agent. Results from this study, however, do not suggest a higher risk of hepatitis B or C infections among donors deferred for "tattooing, ear/body piercing, acupuncture, accidental needlestick, coming into contact with someone else's blood, or taking (snorting) cocaine or any other street drug through one's nose." Similarly, this study did not show an increased risk of hepatitis among donors deferred for travel to malarial areas. The study was unable to assess, however, whether these questions have any impact on other potential blood-borne pathogens that are not currently being screened for during blood collection. Because a majority of deferred donors are unlikely to return for donation following their deferral, many safe blood donors could be saved and retained if the two questions are improved. Analysis of changes in deferral and marker-positive rate associated with UDHQ implementation may be able to show whether the changes will have made a difference.

Larger studies of deferred donors may provide additional important information about the effectiveness of blood donor health history interviews. Such larger studies will almost certainly need to be performed by routine health history interviewers at a significant number of blood centers to provide sufficient statistical power to prove that any of the current donor questions are unnecessary.

**ACKNOWLEDGMENT**

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## APPENDIX

Health history questions targeted:

3. Since the age of 11, have you had yellow jaundice, liver disease, or hepatitis?
4. Have you ever tested positive for hepatitis?
5. In the past 12 months, have you been in close contact with anyone having yellow jaundice or hepatitis, or have you received hepatitis B immune globulin (HBIG)?
7. Have you ever taken clotting factor concentrates for a bleeding problem, such as hemophilia?
12. Have you ever used a needle, even once, to take any illegal or nonprescription drug?
13. Have you taken money or drugs in exchange for sex since 1977?
14. Are you a male who has had sex, even once, with another male since 1977?
17. In the past 12 months, have you traveled outside of the U.S. except Canada, Australia, New Zealand, Japan, or Western Europe, including the British Isles?
18. In the past 12 months, have you received a blood transfusion or an organ or tissue transplant?
19. In the past 12 months, have you had a tattoo, ear/body piercing, acupuncture, accidental needlestick, come into contact with someone else's blood, or taken (snorted) cocaine or any other street drug through your nose?
21. In the past 12 months, have you had or been treated for syphilis or gonorrhea or tested positive for syphilis?
22. In the past 12 months, have you had sex, even once, with anyone who has ever used a needle for illegal or nonprescription drugs?
23. In the past 12 months, have you had sex, even once, with anyone who has taken money or drugs in exchange for sex since 1977?
24. In the past 12 months, have you given money or drugs to anyone to have sex with you?
25. In the past 12 months, have you had sex, even once, with anyone who has taken clotting factor concentrates?
26. In the past 12 months, have you had sex, even once, with anyone who has had AIDS or tested positive for the AIDS virus?
27. Are you a female who, in the past 12 months, has had sex with a male who has had sex, even once, with another male since 1977?
35. Do you have AIDS or have you ever tested positive for the AIDS virus?
37. (a) Were you born in, or have you lived in, Cameroon, Central African Republic, Chad, Congo, Equatorial Guinea, Gabon, Niger, or Nigeria, since 1977? (b) Since 1977, have you received a blood transfusion or medical treatment with a blood product in any of these countries? (c) Have you had sex with anyone who, since 1977, was born in or lived in any of these countries?

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**研究報告の概要**

○心筋灌流測定に使用した汚染した放射性医薬品によるC型肝炎ウイルス(HCV)感染

【背景】放射性医薬品薬局は、心筋灌流測定を含む一般的診断法で使用する放射性医薬品を調製する。核医学画像検査現場でのHCV伝播は、過去に報告されたことはない。

【目的】2004年10月15日に放射性医薬品注射剤を用いて心筋灌流測定を行った患者におけるHCVのアウトブレイクについて調べること。

【デザイン、施設および患者】発生源究明のため、分子疫学調査と心臓外来クリニックおよび放射性医薬品薬局の現地調査を行った。この薬局で2004年10月14～15日に調製された放射性医薬品バイアルから採取した注射剤を投与された患者90名に、血液感染性病原体の検査を求めた。薬局の業務手順を調査し、HCVシークエンス解析を行った。

【結果】3つのクリニックから急性HCV感染患者16名を確認した。いずれの患者も、1つのバイアル(バイアル1)から採取した放射性医薬品注射剤投与を受けた。他の6バイアルの投与を受けた患者59名のうち急性HCV感染を発現した者はいなかった。当該薬局では、バイアル1を調製する12時間前に、HCVおよびヒト免疫不全ウイルス(HIV)に罹患した患者の血液の放射線標識白血球測定を行っていた。この患者から得られたHCVのシークエンスは、当該症例の配列とほぼ同一であった(相同性97.8%～98.5%)。急性HIV感染は確認されなかった。血液の交差汚染の原因と考えられる薬剤部の業務行為には、針の再利用や希釈時のシリンジ再利用、滅菌や血液由来製剤調製の一部の工程で同じ層流フードを使用すること等が挙げられる。

【結論】血液汚染された放射性医薬品から16名の患者がHCVに感染した。当該薬局において無菌操作を破綻させた可能性のある感染源と業務行為が認められた。生物由来製剤を取り扱う放射性医薬品薬局は、適切な無菌操作を行うべきである。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 放射性医薬品注射剤を用いる心筋灌流測定を実施した患者において、血液汚染された注射剤から16名の患者がHCVに感染したとの報告である。輸血後HCV感染の調査には、注射剤の血液汚染など輸血以外の要因について考慮する必要がある。 | HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。 |

ORIGINAL CONTRIBUTION

# Hepatitis C Virus Infections From a Contaminated Radiopharmaceutical Used in Myocardial Perfusion Studies

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**Context** Nuclear pharmacies prepare radiopharmaceutical products for use in common diagnostic procedures, including myocardial perfusion studies. Hepatitis C virus (HCV) transmission has not been reported previously in the setting of nuclear imaging studies.

**Objective** To investigate an outbreak of acute HCV infection identified among patients who underwent myocardial perfusion studies on October 15, 2004, using an injected radiopharmaceutical.

**Design, Setting, and Patients** Outbreak investigation including molecular epidemiology and pharmacy site investigation at outpatient cardiology clinics and a nuclear pharmacy in Maryland. Ninety patients who received injections drawn from select radiopharmaceutical vials prepared on October 14-15, 2004, at a single nuclear pharmacy were offered testing for bloodborne pathogens. Pharmacy procedures were reviewed and HCV quasi species analysis was performed.

**Main Outcome Measures** Hepatitis C virus infection and quasispecies sequence similarity.

**Results** Sixteen patients with acute HCV infection were identified from 3 separate clinics. All patients received radiopharmaceutical injections drawn from a single pharmacy vial (vial 1). None of the 59 tested patients who received doses from 6 other vials had acute HCV infection. Blood from a potential source patient with HCV and human immunodeficiency virus (HIV) infection was processed for a radiolabeled white blood cell study in the pharmacy 12 hours before vial 1 was prepared. The HCV quasispecies sequences from this potential source patient were nearly identical to those from cases (97.8%-98.5% similarity). No acute HIV infections were identified. Pharmacy practices that could have led to blood cross-contamination included reuse of needles and syringes during dilutions and use of common flow hoods for some steps in the preparation of sterile and blood-derived products.

**Conclusions** Sixteen persons acquired HCV infection from a blood-contaminated radiopharmaceutical. The source and practices that could have facilitated breaks in aseptic technique were identified at the pharmacy. Nuclear pharmacies that handle biological products should follow appropriate aseptic technique to prevent contamination of sterile radiopharmaceuticals.

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HEPATITIS C VIRUS (HCV) INFECTION is the most common chronic bloodborne infection in the United States, with an estimated antibody prevalence of 1.6%.[1] Transmission of HCV primarily occurs through percutaneous blood exposure; injection drug use is the most common risk factor.[1] Health care–associated transmission of HCV is thought to be unusual in developed countries but outbreaks primarily attributed to contaminated medications or equipment and breaches in aseptic technique have been reported recently in health care settings in the United States, Europe, and Japan.[2-12] Transmission of HCV in the setting of nuclear imaging studies has not been reported previously.

In 2005, an estimated 19.7 million nuclear medicine procedures were per-

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formed in the United States; more than half of these were myocardial perfusion studies.[13] Myocardial perfusion studies involve the intravenous injection of a radionuclide (eg, thallium 201 or technetium 99m–labeled sestamibi [Tc 99m sestamibi]), which is detected by nuclear imaging. These radiopharmaceutical products are often prepared in commercial nuclear pharmacies and distributed to hospitals or outpatient clinics for administration.[14] Although biological materials are not used in the preparation of radionuclides for myocardial perfusion studies, nuclear pharmacies may process blood for radiolabeled blood cell studies.

In November 2004, 2 older adults with no recognized risk factors for HCV infection were diagnosed with acute HCV infection and reported to a local health department in Maryland. Both patients had symptom onset in early November 2004 and had undergone myocardial perfusion studies at an outpatient cardiology clinic on October 15, 2004. The radiopharmaceutical used was Tc 99m sestamibi, which was prepared by a nuclear pharmacy that also prepared radiopharmaceuticals for other health care facilities in the state. Additional cases of acute HCV infection were subsequently identified among patients who underwent perfusion studies at different clinic sites and who had received Tc 99m sestamibi prepared by the same pharmacy on October 15. The Maryland Department of Health and Mental Hygiene conducted an investigation to determine the mode and extent of HCV transmission.

## METHODS

### Epidemiological Investigation

All patients who underwent a myocardial perfusion study on October 15 and received Tc 99m sestamibi from the same multidose pharmacy preparation vial as the initial cases (vial 1) were tested for evidence of HCV, hepatitis B virus (HBV), and human immunodeficiency virus (HIV) infection. Using an estimate of the maximum number of doses that could have been prepared from the same multidose saline container used to dilute vial 1, HCV, HBV, and HIV testing were recommended for any patient who received Tc 99m sestamibi from the vial prepared before (vial 0), or any of the 5 vials prepared immediately after vial 1 (vials 2-6). A case was defined as evidence of HCV infection (presence of HCV RNA with either symptoms of acute viral hepatitis or elevated serum aminotransferase levels) in a person who underwent a myocardial perfusion study on October 14-15, 2004.

To determine whether other cases of HCV infection might have resulted from exposures to radiopharmaceuticals prepared by other pharmacies or on different dates, enhanced surveillance for acute HCV cases was conducted. Local health departments in Maryland and infection-control practitioners were contacted to request immediate reporting of all suspected cases and other state health departments were notified through outbreak alerts. Cases reported to the Maryland Department of Health and Mental Hygiene were investigated for recent exposure to nuclear imaging studies. Medical records were reviewed to obtain clinical and laboratory information for potential case and source patients. Demographic information was collected from standard case report forms. Predefined categories were used to collect race/ethnicity information on the surveillance case report forms.

A summary of this public health investigation was submitted to the Science Office in the Office of Workforce and Career Development, Centers for Disease Control and Prevention, for research/nonresearch determination. The Office of Workforce and Career Development Science Office determined that the activities involved in the investigation constituted an urgent public health response and did not require submission of a protocol to the institutional review board.

### Nuclear Pharmacy Investigation

A site investigation of the nuclear pharmacy was conducted, including a review of all pharmaceuticals prepared and biologics handled on October 14-15, 2004. The Tc 99m sestamibi and white blood cell (WBC) radiolabeling procedures were observed. Pharmacy employees were screened for anti-HCV, and if positive, were tested for HCV RNA. Employees involved in Tc 99m sestamibi preparation on October 15 or WBC labeling on October 14 were interviewed about their work practices and training.

### Review of Radiopharmaceutical Preparation

From our observations, we determined that for each pharmacy preparation of Tc 99m sestamibi, Tc 99m was drawn into a needle and syringe and added to a prefilled manufacturer's vial of sestamibi powder. Each vial of combined sestamibi and Tc 99m was then heated at 100°C for 10 minutes. Saline drawn from multidose bags or vials was added to dilute the mixture before and after heating. Mixing and dilution were performed by a pharmacist in a laminar flow workstation in a centrally located room of the pharmacy (main room). Following heating, dilution, and assay of each preparation vial of Tc 99m sestamibi solution, individual patient doses (approximately 7-16 per vial) were drawn into syringes by pharmacy technicians in the main room. These prefilled syringes were packaged and delivered to health care facilities for administration.

The WBC radiolabeling procedure was performed by a pharmacist in a designated blood room, adjacent to the main room. For this procedure, whole blood was received and placed in a flow hood to be separated into components. The procedure involved several centrifuge tubes, multiple syringes, and frequent washes of the separated cells using saline from a multidose vial. The procedure also required the pharmacist to leave the blood room carrying the blood-derived preparation to use a hood in the main room where Tc 99m sestamibi and other sterile radiopharmaceuticals were prepared.


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### Laboratory and Source Investigation

Patient and pharmacy employee serum samples were tested for anti-HCV (Ortho HCV, version 3.0 enzyme-linked immunosorbent assay, Ortho-Clinical Diagnostics, Raritan, NJ). Serum samples from patients and potential source patients (including 3 archived specimens that had been stored at −80°C) were tested for the presence of HCV RNA by qualitative polymerase chain reaction (Amplicor HCV test, version 2.0, Roche Molecular Systems, Branchburg, NJ). Genotypes of HCV were determined using previously described methods.[15] Potential source patients with undetectable HCV RNA or an HCV genotype that did not match that of the cases were ruled out, leaving 1 suspected source patient.

Patient serum samples were also tested for hepatitis B surface antigen (HBsAg) (ETI-MAK-2, DiaSorin srl, Saluggia, Italy), total antibodies to the hepatitis B core antigen (Bio-Rad Laboratories, Redmond, Wash), and antibodies to HIV (anti-HIV) (Vironostika HIV-1 Microelisa System, bioMerieux Inc, Durham, NC; and HIV-1/HIV-2 Plus O, Bio-Rad Laboratories). Serum samples that tested positive for HBsAg and total antibodies to the hepatitis B core antigen were tested for IgM antibodies to the hepatitis B core antigen (ETI-CORE-IGMK, DiaSorin, Stillwater, Minn) and antibodies to hepatitis B e antigen (ETI-AB-EBK Plus, DiaSorin). Results of serological or RNA tests performed by licensed clinical laboratories were accepted in place of our testing. Follow-up testing for HBsAg was recommended at 4 and 6 months, and for anti-HIV at 4, 6, and 12 months, postexposure for all cases.

The HCV hypervariable region (HVR1) quasi species were isolated and analyzed for cases, the suspected source patient, and a reference group obtained from the Third National Health and Nutrition Examination Survey (NHANES III).[16] The NHANES III reference group included 5 randomly selected participants with HCV genotype 1a infection whose sequences in the HCV NS5b protein-coding region showed a 95% or higher sequence similarity to that of the suspected source. The HVR1 quasi species were isolated and amplified by limiting dilution polymerase chain reaction and sequenced as described previously.[15] Pairwise analysis was used to calculate the distribution of nucleotide variation by using Pileup and Evolutionary Distance programs in the Accelrys GCG Package (Genetic Computer Group, version 10.3, Accelrys Inc, San Diego, Calif). Sequence analysis by different methods indicated similar clustering of sequences. The final neighbor-joining phylogenetic tree was constructed by using Kimura's 2-parameter model (DNADIST, NEIGHBOR, and DRAWTREE programs of the PHYLIP package, version 3.63, Seattle, Wash),[17] and bootstrap analysis was performed to evaluate the reliability of the phylogenetic tree.[18]

## RESULTS

### Epidemiological Investigation

Sixteen cases were identified; all underwent myocardial perfusion studies on October 15. Cases had perfusion studies performed at 3 unaffiliated clinics (TABLE 1). The median age of cases was 63 years and 14 (88%) were male. Fifteen cases were symptomatic, including 11 who had jaundice. The median (range) time from exposure to first symptom onset was 24 (15-41) days. One case experienced liver failure and died 10 weeks postexposure; the primary cause of death was sepsis. A second case died from a cerebrovascular event 9 months postexposure. All 16 cases had received injections of Tc 99m sestamibi drawn from vial 1 and were the only patients to have received doses from this vial (TABLE 2).

A total of 90 patients received injections drawn from vials 0 through 6. Of 74 patients who received injections drawn from vial 0 or vials 2 through 6, 2 were previously known to have chronic HCV infection. Of the remaining 72 patients, 59 (82%) agreed to be tested for anti-HCV and all were negative; 46 (78%) of 59 patients were additionally tested for HCV RNA and all were negative. The attack rate for new HIV (n=54) or HBV (n=53) infections was 0% among patients tested who received injections drawn from vial 0 or vials 2 through 6.

**Table 1.** Demographic and Clinical Characteristics of Cases (N = 16)

| Characteristic | No. (%) of Cases* |
|---|---|
| Demographic | |
| Age, median (range), y | 63 (45-81) |
| Male sex | 14 (88) |
| White race | 16 (100) |
| Clinical | |
| Facility where patient underwent perfusion study | |
| Clinic A | 8 (50) |
| Clinic B | 1 (6) |
| Clinic C | 7 (44) |
| Symptomatic | 15 (94) |
| Time to symptom onset, median (range), d | 24 (15-41) |
| Jaundice | 11 (69) |
| Hospitalized | 7 (44) |
| Peak alanine aminotransferase ≥2 times upper limit of normal | 15 (94) |
| Documented anti-HCV seroconversion | 5 (31) |

Abbreviation: HCV, hepatitis C virus.
*Unless otherwise indicated.

Of the 16 cases, 12 had sufficient HCV RNA for genotyping and were identified as HCV genotype 1a. One case tested positive for total antibodies to hepatitis B core antigen, HBsAg, and antibodies to hepatitis B e antigen, but was negative for IgM antibodies to the hepatitis B core antigen and had radiographic findings suggestive of chronic HBV infection. The remaining 15 cases had no serological markers of HBV infection, and all 16 cases were anti-HIV negative. Follow-up test results were available for 13 of 15 cases who survived for at least 6 months and 10 of 14 cases who survived for at least 12 months postexposure. At 4 to 6 months postexposure, the 13 cases tested negative for markers of HBV and HIV infection. At 12 months, the 10 cases tested negative for anti-HIV.

### Pharmacy Investigation

Pharmacy records indicated that 33 vials (approximately 300 doses) of Tc 99m sestamibi were prepared on October 15 (FIGURE 1). Vial 0 was the last Tc 99m sestamibi vial prepared on October 14; vial 1 was the first vial prepared on Oc-

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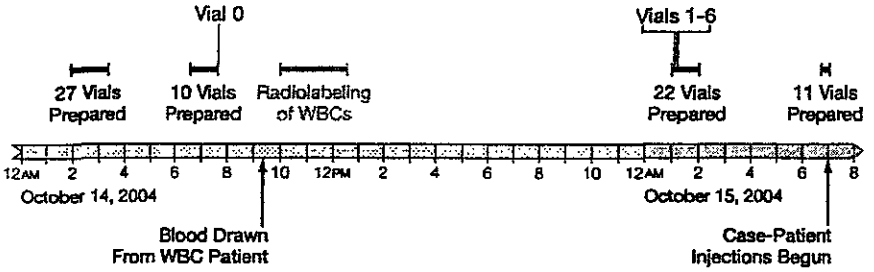
**Table 2.** Selected Vials of Technetium 99m Sestamibi Prepared by the Same Pharmacy, October 14-15, 2004, and Hepatitis C Virus Test Results for Patients Who Received Doses Drawn From These Vials

| | | | | Anti-HCV | | HCV RNA | | |
|---|---|---|---|---|---|---|---|---|
| Date Prepared | Time Prepared | Vial No. | No. of Patient Doses | No. (%) of Patients Tested | No. of Patients Positive | No. of Patients Tested | No. of Patients Positive | Attack Rate, % |
| October | | | | | | | | |
| 14 | 7:36 AM | 0 | 3 | 3 (100) | 0 | 3 | 0 | 0 |
| 15 | 1:05 AM | 1 | 16 | 16 (100) | 15 | 16 | 16 | 100 |
| 15 | 1:06 AM | 2 | 15 | 14 (93) | 0 | 14 | 0 | 0 |
| 15 | 1:07 AM | 3 | 16 | 11 (73)* | 0 | 9 | 0 | 0 |
| 15 | 1:08 AM | 4 | 16 | 13 (81) | 0 | 8 | 0 | 0 |
| 15 | 1:09 AM | 5 | 14 | 9 (69)* | 0 | 5 | 0 | 0 |
| 15 | 1:10 AM | 6 | 10 | 9 (90) | 0 | 7 | 0 | 0 |

Abbreviation: HCV, hepatitis C virus.
*Excludes patients previously known to have chronic HCV infection (n = 2).

**Figure 1.** Vials of Technetium 99m Sestamibi Prepared and Blood Procedure Performed in the Same Nuclear Pharmacy by Time, October 14-15, 2004




WBC indicates white blood cell.

tober 15. The first 6 vials on October 15 were batched and prepared together, with no more than 5 minutes separating their start times (Table 2).

According to pharmacy records, the only preparation involving blood products on October 14-15 was a WBC-radiolabeling procedure performed on October 14, approximately 12 hours before vial 1 was prepared. A syringe containing 50 mL of whole blood was processed to isolate WBCs and to label them with indium (In) 111 for a WBC scan. The pharmacist who performed WBC radiolabeling on October 14 was not involved in the Tc 99m sestamibi preparation on October 15.

Of 28 employees who were working at this pharmacy in October, 27 were located and agreed to be screened for HCV infection. One employee was anti-HCV positive but HCV RNA negative. This employee had been tested previously and was thought to have resolved infection on the basis of past negative HCV RNA test results. The remaining 26 employees were anti-HCV negative, including the pharmacists who prepared the labeled WBCs and vial 1, as well as the technicians who drew the individual doses on October 15. The employee who could not be contacted had stopped working at the pharmacy on October 8.

Upon inspection of the pharmacy, unwrapped syringes with needles attached were noted in radiopharmaceutical preparation areas, including workstations. Recapping of needles was observed during procedures, and was reported as a routine practice by pharmacy workers. During observed procedures, syringes containing radioactive material were discarded immediately following use, but syringes used to add saline were commonly left in workstations and used for several preparations. Employees reported having received training in radiation safety and aseptic technique. Proficiency in aseptic technique was regularly assessed by growth media challenge tests (a sterile preparation of culture media performed and subsequently observed for microbial growth). Although the pharmacy maintained documentation of reported needlestick injuries, several employees claimed that sharps injuries were not always reported to the pharmacy's management.

### Laboratory and Source Investigation

The patient whose blood had been processed on October 14 (WBC patient) was a nursing home resident with a history of HCV, HBV, and HIV infections, who had been hospitalized for altered mental status and urosepsis. The patient was reinjected with the radiolabeled WBCs prepared by the nuclear pharmacy but was discharged before the imaging scan was performed and died approximately 1 week later. Frozen serum specimens that had been collected from this patient in 1996, 1999, and July 2003 were retrieved. Testing determined that all specimens were HCV RNA positive, genotype 1a.

This patient had previously received antiretroviral therapy for HIV infection but had not been treated for HCV infection. Laboratory studies revealed an HIV-1 RNA concentration of 15 104 copies/mL in August 2004, and an HCV RNA concentration of $1.2 \times 10^6$ copies/mL in May 2000. In November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2002, the patient tested negative for HBsAg.

Nine of 16 cases had sufficient HCV RNA for quasi species analysis. Quasi species analyses for HVR1 were performed on specimens from these 9 cases and the WBC patient, and compared with 5 NHANES III participants with genotype 1a infection (FIGURE 2). Cases had 2 to 15 distinct HVR1 sequences that were 98.5% to 100% identical to each other. Eleven distinct HVR1 sequences were characterized from the WBC patient specimen collected in 2003. Similarity among the HVR1 sequences in the WBC patient specimen collected in 2003 and the sequences of the 9 cases ranged from 97.8% to 98.5%. The similarity among HVR1 sequences from the WBC patient specimens collected in 1996, 1999, and 2003 was 95.5% to 99.3%. The relatedness of HVR1 sequences from the specimens of cases and the WBC patient compared with the NHANES III specimens ranged from 79.7% to 89.9%. The sequences from the cases and the WBC patient clustered as a group distinct from those of the NHANES III specimens.

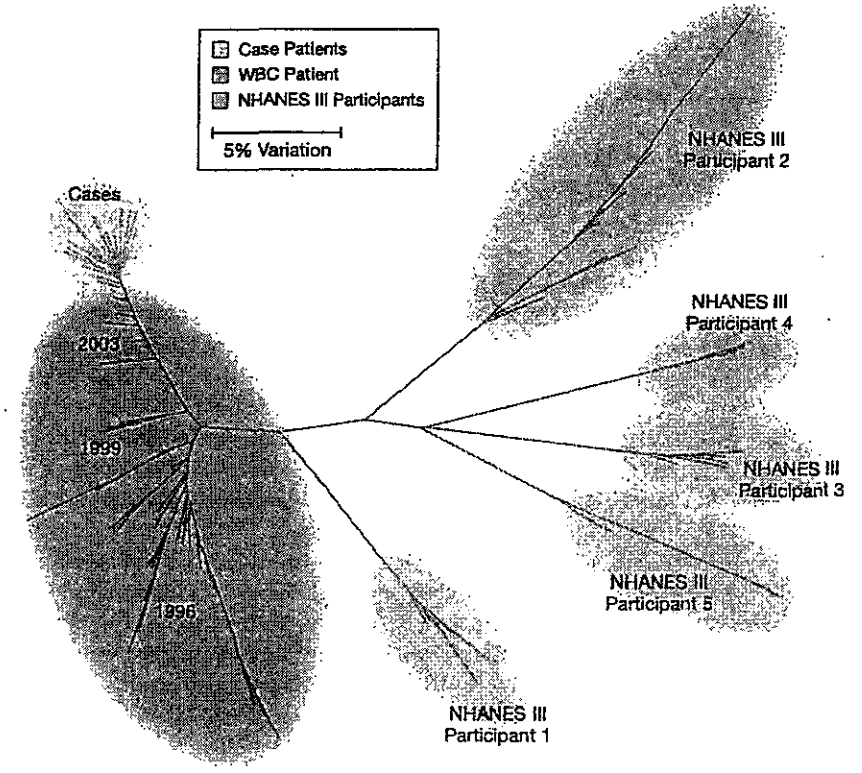
## COMMENT

The findings from this and other recent investigations demonstrate that bloodborne pathogens can be transmitted in any setting where blood exposures occur and aseptic technique is compromised. Health care–related exposures should be considered in the evaluation of patients with acute HCV infection, and clinicians should report these cases to facilitate prompt identification and control of potential outbreaks. This outbreak was detected only after symptomatic patients were reported to a local health department that conducts enhanced HCV surveillance and routinely investigates acute cases. Their investigation of the initial cases identified a common exposure to outpatient cardiology procedures on the same date. The resulting expanded investigation revealed that 16 patients who underwent myocardial perfusion studies at 3 separate clinics acquired HCV infection after receiving Tc 99m sestamibi injections drawn from a single pharmacy preparation vial. Blood from a patient with chronic HCV and HIV infections was the source of HCV transmission, probably through contamination of supplies (syringes or a multidose saline vial) during the preparation of In 111–labeled WBCs on October 14. Although a cost analysis was beyond the scope of this investigation, the costs associated with this outbreak were likely substantial, including those to patients with symptomatic illness that required hospitalization, as well as those incurred by the medical care and public health systems involved. Transmission of HCV from a contaminated radiopharmaceutical has not been described previously.

Multiple lines of evidence support the proposed mode of HCV transmission. Simultaneous transmission to 16 patients occurred in 3 unaffiliated clinics. The attack rate was 100% among patients who received Tc 99m sestamibi from the implicated pharmacy vial and 0% among patients who received doses from the other vials. No pharmacy employee was viremic for HCV. This pharmacy performed no other procedures involving blood products. Furthermore, quasi species analysis demonstrated that the HVR1 variability among the source patient and the cases was less than the variability observed over time within the source patient, as measured at time points spanning 7 years.

Transmission of HCV in health care settings has been linked to failure to adhere to aseptic technique, including reuse of syringes and needles and contamination of multidose medication vials.[2-5,8,12] Although the specific break in aseptic technique that led to transmission was not identified, a number of

**Figure 2.** Unrooted Phylogenetic Tree of Hepatitis C Virus Hypervariable Region Sequences Identified From the White Blood Cell Patient at 3 Different Periods, 9 Cases, and Selected Third National Health and Nutrition Examination Participants




NHANES III indicates Third National Health and Nutrition Examination; WBC, white blood cell.

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observations support the hypothesis that a syringe or multidose saline vial contaminated during the WBC radiolabeling procedure could have been used inadvertently in the Tc 99m sestamibi preparation. First, overlap occurred between areas of the pharmacy where blood components were processed and areas where the Tc 99m sestamibi was prepared. Second, practices that could lead to difficulty distinguishing between used and unused syringes were identified, including unwrapping syringes well in advance of their use, and recapping needles without immediate disposal. Moreover, reuse of syringes was not prohibited. Third, multidose saline vials were frequently used and commonly shared between sterile preparations, and thus could have been a contaminated reservoir.

The exact duration of HCV viability in the environment is unknown. In chimpanzee infectivity studies, HCV in dried plasma at room temperature remained infectious for 16 hours or longer.[19] The timeline that can be constructed from the pharmacy's records provides additional information regarding HCV viability. Because blood was drawn from the WBC patient approximately 15 hours before preparation of the contaminated Tc 99m sestamibi vial, and cases received injections between 6 and 8 hours later, our findings suggest that HCV at room temperature can remain infectious to humans for at least 21 to 23 hours.

Although the source patient was coinfected with HIV, no evidence existed of HIV transmission. Several factors might explain the absence of HIV transmission in a setting in which HCV transmission was highly efficient. Human immunodeficiency virus is likely less stable than HCV in a room temperature environment[19,20]; and the source patient had received antiretroviral treatment in the past, which might have attenuated HIV transmissibility (a consideration supported by the relatively low viral load in August 2004). Although delayed HIV seroconversion among HCV-infected patients has been reported,[21,22] no cases had evidence of HIV infection at up to 1 year postexposure.

Nuclear pharmacies are a specialized form of compounding pharmacy regulated by state pharmacy boards and the US Nuclear Regulatory Commission or its state designee. Approximately 370 nuclear pharmacies operate in the United States; most are centralized suppliers, whereas others are hospital-based.[14,23] Outbreaks of bloodstream infections and meningitis[24-29] have been attributed to microbial contamination of injected products prepared in compounding pharmacies, raising concerns regarding the adequacy of compounding pharmacy standards, training, and oversight.[30-36] According to industry observers, comprehensive courses on sterile compounding are not offered at most colleges of pharmacy and many pharmacists have little training in aseptic manipulation skills.[31-34] Recent surveys of hospital compounding pharmacies have indicated that compliance with quality-assurance guidelines published by the American Society of Health-System Pharmacists remains unacceptably low.[37]

The US Pharmacopeia was recently revised to address pharmaceutical compounding of sterile preparations, including radiopharmaceuticals, and new content has been proposed to address the issue of blood-derived products in nuclear pharmacies.[38,39] However, no specific information on risk for bloodborne pathogen contamination of compounded pharmaceuticals is provided in the Nuclear Pharmacy Compounding Guidelines from the American Pharmaceutical Association, nor in the model rules for nuclear/radiologic pharmacy from the National Association of Boards of Pharmacy.[40,41] Furthermore, few explicit strategies aimed at bloodborne pathogen risk reduction are presented in these guidelines or in the US Pharmacopeia.[38,40,41] The paucity of guidance on blood manipulation within pharmacies was reflected in this outbreak investigation, which found that training on aseptic technique at the nuclear pharmacy was directed at prevention of microbial contamination and growth, and protocols to address bloodborne pathogen risks appeared limited to worker safety issues rather than the risk of blood contamination of compounded radiopharmaceuticals.

All compounding pharmacies should comply with US Pharmacopeia standards and establish policies to ensure sterile equipment and environments, standardized compounding procedures, and training of employees on aseptic technique. Nuclear pharmacies that handle blood products should additionally recognize the risks for blood contamination of radiopharmaceuticals and implement appropriate precautionary measures to prevent such contamination. The safety of parenteral medications and diagnostic pharmaceuticals depends on careful application of aseptic techniques across the entire spectrum of their preparation and administration. The findings from this investigation, as well as other reported outbreaks, underscore a need for heightened awareness and renewed vigilance.

**Author Contributions:** Dr Patel had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.
*Study concept and design:* Patel, Larson, Castel, Farrell, Krick, Blythe, Fiore, Roche.
*Acquisition of data:* Patel, Larson, Castel, Ganova-Raeva, Myers, Roup, Farrell, Edwards, Nainan, Blythe, Roche.
*Analysis and interpretation of data:* Patel, Larson, Castel, Ganova-Raeva, Roup, Farrell, Nainan, Krick, Blythe, Fiore, Roche.
*Drafting of the manuscript:* Patel, Castel, Myers, Nainan, Blythe, Fiore.
*Critical revision of the manuscript for important intellectual content:* Patel, Larson, Castel, Ganova-Raeva, Roup, Farrell, Edwards, Krick, Blythe, Fiore, Roche.
*Statistical analysis:* Patel, Larson, Castel, Nainan, Roche.
*Obtained funding:* Farrell.
*Administrative, technical, or material support:* Patel, Larson, Ganova-Raeva, Myers, Roup, Farrell, Edwards, Nainan, Krick, Blythe, Roche.
*Study supervision:* Patel, Krick, Blythe, Fiore, Roche.
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| 販売名<br>(企業名) | フィブリノゲンHT-Wf （ベネシス） | | | | | |

**研究報告の概要**

チェコ共和国における IVIG 投与患者の血清中における HGV 陽性率を調査し、HGV に関係したリスクを検討した。その病原性は不明であるが、HGV は肝疾患又はリンパ増殖を起こす可能性がある。試験を行った 86 名の IVIG 投与患者の内の 20 名（23%）が、HGV RNA 陽性であった。これは、およそ 4.2%と報告されている一般人口における平均的な感染率、あるいはチェコ共和国の人口比 6%よりも高くなっている。本研究で確認した HGV 陽性反応の割合は、非経口感染のリスクグループの感染率にほぼ相当する。
HGV 陽性患者 20 人の内、3 人（15%）には肝機能検査値の緩やかな上昇が認められ、また 1 人（5%）は慢性リンパ性白血病に罹されていたが、これは IVIG 置換開始前にすでに診断が下されていた。IVIG 投与患者の HGV 感染率は高いが、肝疾患又はリンパ増殖のいずれの徴候とも関係していないと、結論付けることができる。

**使用上の注意記載状況・その他参考事項等**

2. 重要な基本的注意
(1)本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人フィブリノゲンを濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル(TNBP)／ポリソルベート 80 処理、濾過膜処理(ナノフィルトレーション)、凍結乾燥の後、60℃、72 時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| チェコ共和国における IVIG 投与患者の HGV 陽性率が健常人より高かったとの報告である。<br>IVIG投与患者におけるHGV陽性率に関する文献報告を弊社では5報入手しているが、それら文献での陽性率は7.0%(4/57)、12.5%(2/16)、0%(0/4)、0%(0/15)、2.5%(1/40)であり、今回の研究報告での23%はこれら報告と比べるとかなり高い値となっている。本文献では、これら過去の報告を引用しての考察が行われていないため、チェコでの陽性率が高い理由については不明である。<br>HGVは30～60nmの大きさのフラビウイルス科に属するエンベロープウイルスである。万一、原料血漿にHGVが混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

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# Výskyt infekce virem hepatitidy G (HGV) u pacientů léčených intravenózními imunoglobuliny v České republice

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管理部

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## Souhrn

**V naší práci se zabýváme prevalencí výskytu viru hepatitidy G (HGV) v séru pacientů léčených intravenózními imunoglobuliny (IVIG) a hodnotíme případné důsledky plynoucí z této chronické infekce. Přestože patogenicita viru hepatitidy G je sporná, předpokládá se, že může vyvolat jaterní postižení či lymfoproliferaci. Z našeho souboru 86 pacientů byla prokázána přítomnost HGV celkem u dvaceti (23 %). U 3 z nich (15 %), pak byla prokázána lehká elevace jaterních testů. Jeden z HGV pozitivních pacientů trpěl chronickou lymfatickou leukemií (5 %), nicméně toto onemocnění bylo diagnostikováno již před zahájením substituce IVIG. Lze uzavřít, že prevalence infekce HGV je u pacientů léčených IVIG vysoká, ale není spojena se známkami jaterní léze ani lymfoproliferace.**

**Klíčová slova: virus hepatitidy G – intravenózní imunoglobuliny – běžný variabilní imunodeficit, X-vázaná agamaglobulinemie – virus hepatitidy C – virus hepatitidy B.**

## Summary

### Sobotková M., Bartůňková J., Litzman J., Zachová R., Jílek D., Kryštůfková O., Němeček V., Vernerová E., Šedivá A.: Prevalence of Hepatitis G Virus (HGV) in Intravenous Immunoglobulin Recipients in the Czech Republic

**The prevalence of hepatitis G virus (HGV) in the serum of intravenous immunoglobulin (IVIG) recipients was studied and risk related to HGV positivity was considered. Although its pathogenicity is unclear, HGV is likely to cause liver disease or lymphoproliferation. Twenty (23 %) of 86 tested IVIG patients were HGV RNA positive. Of the HGV positive patients, three (15 %) showed mild elevation of liver enzymes and one (5 %) was diagnosed with chronic lymphatic leukaemia prior to the institution of IVIG replacement. It can be concluded that the HGV prevalence among IVIG recipients is high but is not associated with signs of either liver disease or lymphoproliferation.**

**Key words: hepatitis G virus – intravenous immunoglobulins – common variable immunodeficiency – X-linked agammaglobulinemia – hepatitis C virus – hepatitis B virus.**

Virus hepatitidy G byl objeven roku 1995 dvěma laboratořemi nezávisle na sobě. V tomto roce se v laboratořích Abbot, Boehringer Mannheim a Genelabs podařilo vyklonovat genetický materiál dvou virů, které byly nazvány HGV a HGBV-C. Základ pro objevení HGBV-C položil již v roce 1967 Friedrich Deinhart, který infikoval sérem pacienta s akutní hepatitidou nejasné etiologie opice – tamariny. Tímto pacientem byl 34 letý chicagský chirurg s iniciálami G.B. (odtud jedno z pojmenování viru). Infekce byla přenášena parenterálně na další generace opic, až se v roce 1995 podařilo v jejich séru prokázat 3 specifické nukleotidové sekvence – HGBV-A, HGBV-B, HGBV-C. Následně se ukázalo, že pouze HGVB-C je virem lidským. Viry HGBV-C a HGV jsou vzhledem ke značné homologii aminokyselinových sekvencí a nukleotidů považovány za dva genotypy téhož viru [4].





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Virus se řadí do čeledi *flaviviridae*, stejně jako virus hepatitidy C. Jeho RNA má 9400 nukleotidů, virová partikule bývá velká asi 30–60 nm a je opouzdřena. Přenos se děje cestou parenterální, pohlavním stykem a slinami. Rovněž je zvažována transmise vertikální a přenos z matky na dítě v průběhu porodu. Inkubační doba činí pravděpodobně 30–120 dní.

Patogenicita viru je sporná a zatím nejsou jednoznačné důkazy, že by způsoboval nějakou přesně identifikovatelnou jaterní či mimojaterní chorobu. Přesto se předpokládá, že může způsobit lehkou akutní hepatitidu, chronickou hepatitidu či perzistentní infekci. Jaké buňky jsou virem infikovány, stále není jasné, ale podezření padá zejména na buňky hemopoetické a lymfoidní, především CD 4 pozitivní T lymfocyty [15]. Zvažuje se proto i možnost, že by virus mohl vyvolat lymfoproliferativní onemocnění [17].

Většina pacientů infikovaných HGV má jen lehce zvýšené hladiny aminotransferáz. Klinický a histologický obraz hepatitidy je zpravidla přítomen jen při koinfekci s dalšími hepatotropními viry. Z hlediska terapeutických možností bylo prokázáno, že při podávání interferonu alfa zpravidla dochází k vymizení HGV ze séra pacienta, nicméně krátce po ukončení terapie se hodnoty HGV RNA vrací na hodnoty před terapií [3].

Výskyt HGV v séru v běžné populaci je asi 3–10 %, ve skupinách s rizikem parenterálního přenosu je uváděno riziko více než 25 % [16].

Vzhledem k tomu, že intravenózní imunoglobuliny jsou připravovány z poolů plazmy od velkého počtu dárců, lze přítomnost HGV v těchto produktech předpokládat. V průběhu jejich výroby jsou však vkládány kroky, které mají zajistit inaktivaci virů (pasterizace, S/D – solvent/detergent, ošetření proteolytickými enzymy) [2, 6, 12].

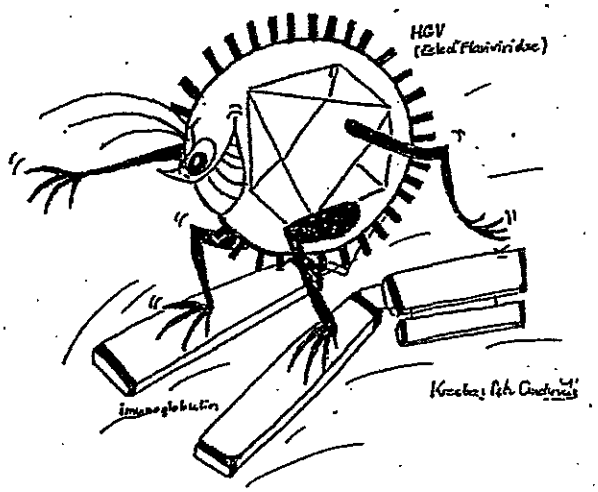
V naší studii jsme analyzovali výskyt infekce virem HGV u pacientů léčených pravidelnými infuzemi imunoglobulinů a známky jaterní léze a případné lymfoproliferace.

## Metody

*Soubor pacientů*

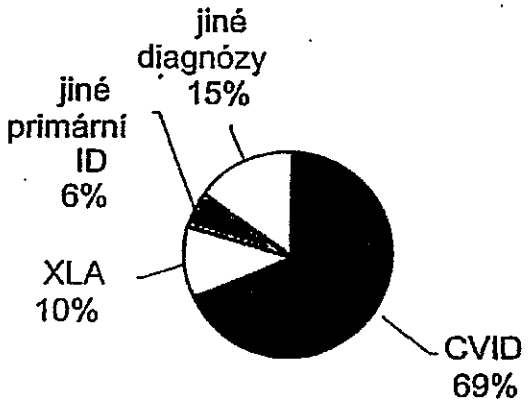
Soubor zahrnuje 86 pacientů z fakultní nemocnice Motol (22 pacientů), Revmatologického ústavu v Praze (11 pacientů), Fakultní nemocnice u sv. Anny v Brně (46 pacientů) a Zdravotního ústavu se sídlem v Ústí nad Labem (7 pacientů), vyšetřených na toto agens v letech 2002 a 2003.

Důvodem k aplikaci intravenózních imunoglobulinů (IVIG) byla ve většině případů diagnóza CVID (běžný variabilní imunodeficit). Z této indikace byly imunoglobuliny podávány 59 pacientům. Dalším důvodem k substituční léčbě byla u 9 pacientů agamaglobulinémie Brutonova typu (XLA). Pět dalších pacientů mělo jiné formy primárních imunodeficiencí: u 3 se jednalo o různé typy hypogamaglobulinémie (blíže nespecifikova-




né), v jednom případě o hyper IgM syndrom (HIMS) a dále pak 1krát syndrom Holtové-Oramův (kombinovaný imunodeficit zejména buněčného typu s projevy recidivujících infekcí a vývojové vady). U 11 dalších pacientů byly IVIG podávány z indikace sekundární protilátkové imunodeficience. Ve dvou případech se IVIG aplikovaly z imunosupresivní indikace (vaskulitida – syndrom Vogt-Konayagi-Harada a 1krát jizvící pemfigoid s očním postižením).

Doba pravidelné aplikace IVIG pacientům byla od 1 do 19 let, v průměru pak asi 5,9 roku (viz graf 1).




**Graf 1.** Zastoupení jednotlivých diagnóz v našem souboru

**Fig. 1.** Distribution of study patients by diagnosis

*Laboratorní vyšetření*

Cílem práce bylo zejména zmapovat výskyt viru hepatitidy G u pacientů na pravidelné terapii IVIG. Vyšetřili jsme jednorázově přítomnost HGV v séru pacientů. Kromě výskytu HGV jsme hodnotili i přítomnost viru hepatitid B (HBV) a C (HCV). HGV a virus hepatitidy C (HCV) byly vzhledem k malé pravděpodobnosti protilátkové odpovědi u pacientů s imunodeficity prokazovány metodou polymerázové řetězové reakce (PCR ) (SZÚ – Národní referenční laboratoř pro hepatitidy) [7]. Infekci HBV jsme hodnotili podle přítomnosti HBsAg. Známky jaterního poškození jsme posuzovali podle aktivity aminotransferáz (AST, ALT), aktivity GMT, ALP a hladiny bilirubinu v séru. Jejich stanovení bylo prováděno při odběru krve na vyšetření HGV a jako známku jaterního poškození jsme hodnotili elevaci kteréhokoli z těchto parametrů. Anamnesticky a ze zdravotnické dokumentace jsme zjišťovali případný výskyt lymfoproliferace.





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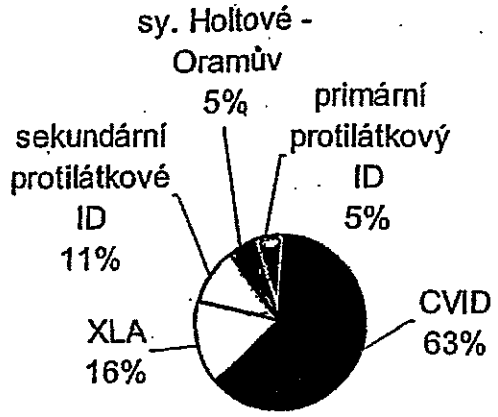


Graf 2. Zastoupení jednotlivých diagnóz u HGV pozitivních pacientů

Fig. 2. Distribution of HGV positive patients by diagnosis

## Výsledky

*Výskyt hepatotropních virů v séru*

Virus hepatitidy G byl nalezen u 20 pacientů z námi sledovaného souboru. U žádného z nich však nebyl v séru současně prokázán HCV ani HBV. HGV pozitivita byla tedy u 23 % námi sledovaných IVIG příjemců.

Virus hepatitidy B jsme prokázali v jednom případě, kdy se však jednalo o pozitivitu již před zahájením terapie IVIG a opět se nejednalo o koinfekci s HGV ani HCV. Celkem u 75 pacientů jsme HBV neprokázali a 10 pacientů nebylo na toto agens vyšetřeno.

Virus hepatitidy C byl prokázán u jednoho pacienta, kdy byla infekce přítomna opět již před zahájením IVIG. U 79 pacientů nebyla infekce HCV prokázána a 6 nebylo na toto agens vyšetřeno.

*Zastoupení HGV v séru na jednotlivých participujících pracovištích*

Na naší studii spolupracovala celkem 4 pracoviště. Ze 7 vyšetřovaných pacientů z Ústí nad Labem byla HGV pozitivita prokázána u 3, tzn. asi u 42 %. Ve skupině pacientů z Revmatologického ústavu v Praze pak byl HGV prokázán v séru 2 pacientů z 9 vyšetřených, tzn. u 22 %. Z 22 pacientů z Ústavu imunologie 2. lékařské fakulty univerzity Karlovy a fakultní nemocnice Motol se HGV vyskytl v séru 5 pacientů, což představuje asi 22 %. Pokud jde o pacienty z brněnského pracoviště, HGV byl prokázán celkem u 10 vyšetřených pacientů z celkového počtu 46. To představuje asi 21 %. Lze tedy uzavřít, že prevalence infekce HGV je prakticky na všech pracovištích shodná, v případě Ústí nad Labem je zkreslena malým počtem pacientů.

*Výskyt HGV pozitivity u jednotlivých diagnóz*

Z celkového počtu 20 HGV pozitivních pacientů probíhala substituce IVIG ve 12 případech z důvodu diagnózy CVID, 3 z nich měli diagnózu XLA a 1 jiný primární protilátkový imunodeficit. Dále byl HGV pozitivní i pacient léčený imunoglobuliny pro jizvící pemfigoid, 2 pacienti se sekundárními protilátkovými imunodeficiencemi a pacient se syndromem Holtové-Orama (viz graf 2). Je tedy zřejmé, že základní diagnóza pravděpodobně neovlivňuje vnímavost k infekci virem HGV.

*Známky jaterního poškození*

Vzhledem k tomu, že se u HGV předpokládá možnost vyvolat u svých nosičů různý typ jaterního poškození, byla dalším sledovaným parametrem tato eventualita. Elevace jaterních testů byla zachycena u 21 pacientů. Z toho u 3 pacientů byl v séru prokázán HGV a u jednoho HCV. Celkem 17 pacientů tedy jevilo známky jaterního postižení, které však nesouviselo s infekcí HGV, HBV ani HCV.

*Výskyt lymfoproliferací u pacientů s HGV*

Vzhledem k výše uváděné suspekci na to, že by HGV mohl být lymfotropní, hodnotili jsme u pacientů také přítomnost či nepřítomnost lymfoproliferací. U námi sledovaných pacientů s HGV pozitivitou byla lymfoproliferace (CLL – chronická lymfatická leukemie) příčinou klinického imunodeficitu, pro který byla substituce IVIG prováděna. U ostatních 19 pacientů, u nichž byla přítomnost HGV prokázána, se lymfoproliferace nevyskytla.

Tab. 1. Základní charakteristika souboru

Table 1. Basic characteristics of study patients

| Důvod k aplikaci IVIG | Celkem pacientů | VHG prokázáno | VHC prokázáno | HBsAg prokázáno | Elevace jaterních testů | Přítomnost lymfoproliferace |
|---|---|---|---|---|---|---|
| CVID | 59 | 12 | 1 | 0 | 13 | 0 |
| XLA | 9 | 3 | 0 | 0 | 0 | 0 |
| Jiné primární ID | 5 | 3 | 0 | 0 | 2 | 0 |
| Sekundární ID | 11 | 2 | 0 | 1 | 3 | 1 |
| Autoimunity | 2 | 1 | 0 | 0 | 2 | 0 |





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## Diskuse a závěr

Cílem naší práce bylo monitorovat výskyt viru hepatitid G u pacientů na substituční terapii intravenózními imunoglobuliny v České republice a možné důsledky plynoucí z infekce HGV. Přesto, že zatím nebyla jednoznačně prokázána patogenicita HGV, předpokládá se u něj schopnost poškození jaterního parenchymu a hypoteticky i možnost vyvolat lymfoproliferaci. V našem souboru 86 pacientů ze 4 českých pracovišť jsme proto nejen hodnotili přítomnost tohoto viru v séru, ale i případné známky jaterního postižení a možné lymfoproliferace. Souhrnné výsledky viz tabulka 1.

U celkem 20 našich pacientů jsme prokázali HGV, což odpovídá asi 23 % vyšetřených. Tato frekvence výskytu je podstatně vyšší než v běžné populaci, kde se uvádí výskyt v průměru kolem 4,2 % postižených, respektive asi 6 % v populaci ČR [8]. Námi zjištěná HGV pozitivita tedy zhruba odpovídá skupině s vysokým rizikem parenterálního přenosu.

Na druhou stranu je třeba říci, že v našem souboru HGV pozitivních pacientů byl pouze jeden s lymfoproliferací, který měl toto onemocnění již před zahájením substituce imunoglobuliny a jen u 3 pacientů s HGV pozitivitou byly přítomny známky lehké elevace jaterních testů. Není bez zajímavosti, že jeden náš HGV pozitivní pacient absolvoval v průběhu studie terapii interferonem alfa z důvodu rozsáhlých veruk a nekrotických lézí v obličeji při kombinovaném imunodeficitu s poruchou sekrece interferonu (syndrom Holtové-Oramův). Při kontrole asi za 6 měsíců po zahájení této léčby došlo k vymizení nukleové kyseliny HGV z jeho séra. Vzhledem k tomu, že tato terapie dosud pokračuje, nelze zatím posoudit, zda bude tento efekt trvalý.

Závěrem můžeme poznamenat, že infekce virem hepatitidy G je u příjemců IVIG vysoká, ale není spojena s klinickými ani laboratorními známkami jaterního poškození ani dosud nebyla příčinou lymfoproliferace.

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112
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# チェコ共和国における免疫グロブリン静注患者の G 型肝炎ウイルス（HGV）感染について

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## 要約

本研究では、免疫グロブリン静注（IVIG）投与患者の血清中の、G 型肝炎ウイルス（HGV）感染率をテーマとし、この慢性感染によって起こりうる現象を分析している。G 型肝炎ウイルスの病原性には議論の余地があるものの、肝機能障害およびリンパ増殖を引き起こす可能性がある、と考えられている。86 人の患者集団のうち、HGV の感染が認められたのは全部で 20 人（23%）である。そのうち 3 人（15%）には、肝機能検査値の緩やかな上昇がみとめられた。HGV 陽性患者のうち 1 人（5%）は、慢性リンパ性白血病に冒されていたが、これは IVIG 置換開始前にすでに診断が下されていた。結論として、IVIG 投与患者の HGV 感染率は高いが、肝機能障害の兆候やリンパ増殖とは関連性がないということが言える。

キーワード：G 型肝炎ウイルス、免疫グロブリン静注療法、分類不能型免疫不全、X 連鎖無ガンマグロブリン血症、C 型肝炎ウイルス、B 型肝炎ウイルス

G 型肝炎ウイルスは 1995 年、2 つの研究所でそれぞれ別々に発見された。この年、アボット、ベーリンガー・マンハイム、ジェネラブスの各研究所が、2 つのウイルスの遺伝物質を無性生殖させることに成功し、それぞれ HGV、HGBV-C と命名された。HGBV-C 発見の土台は、1967 年にフリードリヒ・ダインハルトが、病因不明の急性肝炎にかかった患者の血清を使用し、サルの一種であるタマリンに感染させたことによって、すでに築かれていた。この患者とは 34 歳のシカゴの外科医で、イニシャルは G・B である（ウイルスの名前の一部はここからとられた）。ウイルスは非経口的にサ

ルの親から子へと感染していき、1995年になってようやく、このサルの血清中の、3つの特定ヌクレオチド配列—HGBV-A、HGBV-B、HGBV-C の存在を証明することに成功した。さらに、ヒトに感染するのは HGBV-C のみであることも実証された。HGBV-C および HGV は、アミノ酸配列とヌクレオチドの相同性が明らかであることから、同一ウイルスの2つの遺伝子型であると考えられている[4]。

このウイルスはC型肝炎ウイルスと同様、*flaviviridae* 科に属する。RNA（リボ核酸）は9400個のヌクレオチドを有し、ウイルスの粒子は通常30～60nmの大きさで、形状はカプセル状である。感染経路としては、非経口感染、性感染および唾液による感染がある。同時に垂直感染、出産を通じての母子感染も、可能性が指摘されている。潜伏期間は30～120日間とされている。

このウイルスの病原性には議論の余地があり、現在のところ、正確に識別可能な肝臓疾患および肝外疾患を引き起こすという明らかな証拠はない。にもかかわらず、軽度の急性肝炎や慢性肝炎、持続性感染を引き起こす可能性はあると予測されている。どの細胞が感染するのかは未だに解明されていないが、特に造血細胞とリンパ細胞、なかでも CD4 陽性 T リンパ球ではないかと考えられている[15]。そのため、このウイルスがリンパ増殖疾患を引き起こす可能性もあるのではないかと議論されている[17]。

HGV に感染した患者の多くに、アミノトランスフェラーゼ値のわずかな上昇がみられる。臨床的、また組織学的な肝炎の兆候は、他の肝炎ウイルスとの重感染がある場合のみ現れる。治療法の観点からは、インターフェロン・アルファを投与すると、通常は患者の血清中の HGV が消滅するが、治療を中止すると間もなく、HGV RNA の値が治療前の数値まで戻ることが実証されている[3]。

一般人口における血清中の HGV 感染率は約 3～10％であるが、非経口感染のリスクを保持する集団では、感染率は25％を超えることが報告されている[16]。

免疫グロブリンが、多数の供血者の血漿プールから製造されていることを考慮すれば、その中に HGV が存在する可能性も予測できる。しかしながらグロブリンの製剤過程には、ウイルスの不活性化のための処理（低温殺菌、S/D 処理—有機溶媒／界面活性処理、タンパク質分解酵素処理）が組み込まれている[2, 6, 12]。

本研究では、免疫グロブリン静注療法を定期的に受けている患者の HGV 感染状態および、肝機能障害とリンパ増殖の兆候について、分析を行った。

## 方法

### *患者集団*

患者集団は、医学部附属モトル病院（22人）、プラハ・リウマチ学研究所（11人）、ブルノ・聖アナ医学部附属病院（46 人）、ウースチー・ナド・ラベム医療研究所（7人）の計86人で構成され、2002年および2003年に、G型肝炎ウイルスに関する調査を受けた。

免疫グロブリン静注（IVIG）投与の理由としては、CVID（分類不能型免疫不全症）の治療が最も多く、59 人の患者がこれに当てはまる。その他の理由としては、ブルトン型無ガンマグロブリン血症（XLA）で、9 人が該当する。さらに別の 5 人は、別の形態の原発性免疫不全症患者で、そのうち 3 人は、それぞれ別のタイプの低ガンマグロブリン血症（詳細は特定されていない）、1 人は高 IgM 症候群（HIMS）、残りの 1 人はホルト・オラム症候群（再発性感染および発育障害をともなう、特に細胞性の複合免疫不全症）で治療を受けていた。さらに 11 人の患者は、続発性抗体免疫不全の治療のため、IVIG の投与を受けていた。その他、2 人は免疫抑制状態の兆候（脈管炎—フォークト-小柳-原田症候群）、1 人は目の機能障害をともなう瘢痕性類天疱瘡のために IVIG の投与を受けていた。

患者に対する IVIG の定期投与期間は 1 年から 19 年まであり、平均約 5.9 年になる（図 1 を参照）。

＜イラスト内＞
① HGV（*flaviviridae* 科）
② 免疫グロブリン
③ （判読不能）

*臨床検査*

研究の目的は、主に定期的に IVIG 投与を受けている患者の、G 型肝炎ウイルスの感染状態を調査することであった。そのために、患者の血清中の HGV の有無を 1 回に限って検査した。HGV のほかに、B 型肝炎ウイルス（HBV）および C 型肝炎ウイルス（HCV）の有無も調べた。HGV および C 型肝炎ウイルス（HCV）は、免疫不全症患者の抗体反応の可能性が低いことを考慮し、ポリメラーゼ連鎖反応（PCR）の手法を用いて実証された（国立医療研究所—国立肝炎比較研究室）[7]。HBV に感染しているかどうかは、HBsAg の有無によって判断した。肝機能障害の兆候は、アミノトランスフェラーゼ（AST、ALT）の活性、GMT、ALP の活性および、血清中のビリルビン量によって判定を行った。これらの物質は、HGV 感染調査の採血時の量を基準とし、いずれかの値が上昇した場合は、肝機能障害の兆候であると判断した。また、既往症の記録および医療カルテをもとに、リンパ増殖の有無を確認した。

**調査結果**

*血清中の肝炎ウイルスの有無*

G 型肝炎ウイルスは、本研究で調査した 20 人の患者において感染が認められた。しかしながら、同時に血清中に HCV または HBV が見つかった患者は 1 人もいなかった。本研究で調査した IVIG 投与患者の 23％が、すなわち HGV 陽性であった。

患者のうち1人はB型肝炎に感染していたが、この患者はIVIG投与開始前にすでに陽性判定を受けており、HGVやHCVとの重感染ではない。あわせて75人の患者がHBV陰性と判定され、10人はHBVの感染調査を受けていない。

C型肝炎ウイルスは1人の患者で見つかったが、これもIVIG投与開始前にすでに感染が確認されていた。79人の患者がHCV陰性と判定され、6人はHCVの感染調査を受けていない。

*本研究に参加した各研究機関における、血清中のHGV感染の内訳*

本研究では、あわせて4つの研究機関の協力を得た。ウースチー・ナド・ラベムの7人の患者のうち、HGV陽性の判定を受けたのは3人で、約42%にあたる。プラハ・リウマチ学研究所の患者グループでは、調査対象となった9人のうち2人、すなわち22%の血清中にHGVが確認された。カレル大学第2医学部免疫学研究所と、医学部附属モトル病院のあわせて22人の患者のうち、血清中にHGVが確認されたのは5人で、これは約22%にあたる。ブルノの研究所の患者集団では、調査を受けたあわせて46人の患者のうち、10人にHGV陽性が確認された。これは約21%に相当する。すなわち、HGVの感染率はすべての研究機関でほぼ一致しており、ウースチー・ナド・ラベムの場合はその率が他より少し低くなっていると言うことができる。

*各診断内容別のHGV陽性患者の分布*

HGV陽性と判定されたあわせて20人の患者のうち、12人はCVID、3人はXLA、1人はその他の原発性抗体免疫不全症との診断を受けたために、IVIGの投与を受けていた。その他、1人のHGV陽性患者は瘢痕性類天疱瘡、2人は続発性抗体免疫不全症、さらに1人はホルト・オラム症候群のためにそれぞれ免疫グロブリン療法を受けていた（図2参照）。すなわち、元々の診断がHGVウイルス感受性に影響を及ぼすことはないことが明らかであると言える。

*肝機能障害の兆候*

HGVの感染者においては、さまざまな形態の肝機能障害を併発する可能性が予測されることから、この点についても調査が行われた。肝機能検査値の上昇は、21人の患者において確認された。そのうち3人の患者の血清中にHGVが、1人の血清中にHCVがそれぞれ確認された。すなわち、あわせて17人の患者に肝機能障害の兆候がみとめられたが、HGV、HBVまたはHCV感染との関連性はなかった。

*HGV陽性患者のリンパ増殖の有無*

前述したように、HGVがリンパ球向性ウイルスである疑いがあったため、患者のリンパ増殖の有無についても調査を行った。本研究で調査した患者集団おいて、HGV陽性と判定された患者については、リンパ増殖（CLL—慢性リンパ性白血病）による

免疫不全の臨床症状が、IVIG 投与開始の理由となったケースがあった。HGV 陽性と判定されたその他の 19 人については、リンパ増殖は確認されなかった。

## 考察および結論

本研究の目的は、チェコ共和国における、免疫グロブリン静注投与患者の G 型肝炎の感染状態および、HGV 感染によって起こりうる現象を調査することであった。現在に至るまで、HGV の病原性は明確に実証されてはいないものの、肝臓柔組織の機能障害を引き起こす可能性があること、また仮説ではあるが、リンパ増殖を引き起こす可能性もあることが予測されている。そのため、チェコ国内の 4 つの研究機関、86 人からなる本研究の患者集団では、血清中の HGV ウイルスの有無のみならず、肝機能障害の兆候の有無、またリンパ増殖の有無に関しても調査を行った。総合的な結果については、表 1 を参照されたい。

本研究で調査したうち、20 人の患者が HGV 陽性と判定されたが、これは調査を受けた総患者数の約 23%にあたる。これは、およそ 4.2%と報告されている一般人口における平均的な感染率、あるいはチェコ共和国の人口比 6%よりも高くなっている[8]。本研究で確認した HGV 陽性反応の割合は、非経口感染のリスクグループの感染率にほぼ相当する。

一方で、本研究で HGV 陽性と判定された患者のうち、リンパ増殖が確認されたのはただ 1 人で、しかも免疫グロブリン投与開始前にすでにその診断を受けていたこと、また HGV 陽性患者のうち、肝機能検査値の緩やかな上昇が認められたのは 3 人だけであったことを述べておく必要がある。HGV 陽性患者のうち 1 人は、調査中にインターフェロン分泌異常をともなう複合免疫不全症（ホルト･オラム症候群）による、顔面の広範囲にわたるいぼ状突起物と壊死性障害のため、インターフェロン・アルファ療法を受けていたことも、言及に値する。この治療の開始後約 6 ヶ月目に検査を行ったところ、この患者の血清中の HGV 核酸が消滅していた。治療が現在も継続されていることを考慮すると、この効果が持続するかどうかは今のところ断定することができない。

最後に、G 型肝炎ウイルスの感染率は IVIG 投与患者においては高くなるが、肝機能障害の臨床的・検査結果的兆候とは関連性がなく、今のところリンパ増殖の原因としては確認されていないことを付け加えておきたい。

*本研究は、プロジェクト VZ MSM 0021620812 および 7981-3（GACR）の一環として実施された。*

参考文献

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①その他の診断内容　15%、その他の原発性免疫不全症　6%、XLA　10%、CVID 69%
②図 1：本研究対象患者の診断の内訳
③CVID　63%、原発性抗体免疫不全症　5%、ホルト・オラム症候群　5%、続発性抗体免疫不全症　11%、XLA　16%
④図 2：HGV 陽性患者の診断の内訳
⑤表 1：患者集団の基本的特性
⑥IVIG 投与の理由、患者総数、HGV 陽性、HGV 隠性、HBsAG 陽性、肝機能検査値の上昇、リンパ増殖あり
⑦CVID、XLA、その他の原発性免疫不全症、続発性免疫不全症、自己免疫疾患

別紙様式第 2-1

別紙 3-1

医薬品
医薬部外品　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 9 月 29 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Guideline on validation of immuno-assay for the detection of antibody to human immunodeficiency virus (anti-HIV) in plasma pools. European Medicines Agency (2006). | 公表国<br>米国 | |
| 販売名（企業名） | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 調査書類の評価中に見受けられた，プール血漿の抗ヒト免疫不全ウイルス (抗 HIV) 検査におけるバリデーションの不備のある点を改善するためにこのガイドラインが作成された。抗 HIV イムノアッセイ法は血漿分画製剤の原料となるプール血漿の抗 HIV-1 および抗 HIV-2 抗体を検出する定性試験である。しかし，市販の抗 HIV 検出キットは個人供血サンプルに対してのみバリデーションされている。したがって，血漿分画製剤用プール血漿における検査・バリデーションが必要となる。しかし，プール血漿の血清学的検査は，ウイルス感染の安全性の保証を意味するのではなく，GMP ガイドラインに対する重大な不備を検出する方法とみなされるべきである。市販キットのバリデーションとは，頑健性 (信頼性) はもちろん，特異性と検出感度 (抗原の検出限界値やカットオフ値) の決定を含む多段階プロセスである。加えて，分析法内・分析法間での変動も多数の方法を用いて行うべきである。試験方法は，準備，保管管理，試験，再試験，及び判定の手順に関する情報を含む標準作業手順書 (SOPs) で文書化されなければならない。また，検査キットに非依存性の弱い陽性コントロール (個人供血のカットオフ値の 2-3 倍) を試験ごとに組み入れるべきである。陽性サンプルは，複数の異なる抗原を用いてバリデーションされたイムノアッセイで陰性と実証されない限りは，陽性と見なすべきである。核酸増幅検査 (NAT) は陰性を実証する方法としては使用不可能である。 | BYL-2007-0242 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| EMEA から発行された本ガイダンスは，血漿分画製剤に使用されるプール血漿の HIV-1 および HIV-2 抗体スクリーニングの改善と標準化を目的としている。EMEA は，個人供血の試験用に開発された市販の抗 HIV 検出キットをプール血漿に適応する場合，バリデーションを完全に行うよう勧告している。バリデーションされた確認法は陽性サンプルの再試験に対しても実施すべきであると考える。また，医薬品市販承認取得者と血漿マスターファイル所持者はこのガイドラインに照らし合わせてプール血漿の試験法のバリデーションを検討する必要がある。 | 本ガイドラインに記載された重要事項に対して，弊社の血漿分画製剤に使用されるプール血漿は，既存のバリデーションで適用できていることが確認されていることから，現時点では新たな対応は必要ない。 |



(13)

European Medicines Agency
*Pre-authorisation Evaluation of Medicines for Human Use*

London, 21 September 2006
EMEA/CHMP/BWP/298388/05

**COMMITTEE FOR MEDICINAL PRODUCTS FOR HUMAN USE (CHMP)**

# GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS (ANTI-HIV) IN PLASMA POOLS

| | |
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**GUIDELINE ON VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS (ANTI-HIV) IN PLASMA POOLS**

**TABLE OF CONTENTS**


1. SCOPE ..... 3
2. SELECTION OF THE TEST KIT(S) ..... 4
3. VALIDATION ..... 4
4. QUALITY ASSURANCE ..... 5
5. CONFIRMATION STRATEGIES ..... 6
6. IMPLEMENTATION OF THIS GUIDELINE ..... 6




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## VALIDATION OF IMMUNOASSAY FOR THE DETECTION OF ANTIBODY TO HUMAN IMMUNODEFICIENCY VIRUS (ANTI-HIV) IN PLASMA POOLS

### Introduction

Immunoassays for the detection of antibodies to Human Immunodeficiency Virus (anti-HIV) are qualitative tests for the presence of anti-HIV in pooled plasma for fractionation. The validation requirements are laid down in the following documents:

- The test is considered to be a qualitative limit test for the control of impurities. Therefore, according to the "Note for guidance on validation of analytical procedures: definitions and terminology (CPMP/ICH/381/95)", ICH topic Q2A, the two characteristics regarded as the most important for validation of the analytical procedure are specificity and the detection limit. However, the note for guidance adds "those validation characteristics are regarded as the most important, (...) but occasional exceptions should be dealt with on a case-by-case basis" and Q2A requires that robustness needs to be considered.
- The Ph. Eur. Monograph 01/2005:0853 "Human plasma for fractionation" requires the use of anti-HIV test methods of suitable sensitivity and specificity for plasma pool testing.
- The "Note for guidance on plasma derived medicinal products" (CPMP/BWP/269/95, 3.2.2) specifies that the sensitivity of the test in relation to pool size has to be stated. The intention of the test is defined to be a safeguard against errors in testing or pooling.
- The Ph. Eur. chapter 2.7.1 "Immunochemical methods" requires the use of international reference material. Furthermore, the chapter suggests the use of commercial assay kits.
- The GMP Guide (Volume 4, Chapter 6, 6.21) as well as ISO 17025 (4.6.2) require critical reagents to be under control.

In accordance with these guidelines, the validation characteristics are described as:

- Specificity is the ability to unequivocally assess antibodies to HIV in the presence of other components, which may be expected to be present.
- The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample, which can be detected but not necessarily quantitated as an exact value. In the context of plasma pool testing for anti-HIV, the detection limit should be expressed as endpoint dilution titre(s) of well-characterised positive samples.
- The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal performance.

### Validation Guidelines

### 1. SCOPE

Anti-HIV assay kits, for single donation purposes, marketed in Europe are CE-marked devices and are classified in Annex II A of the Directive 98/79/EC on in vitro diagnostic medical devices. The kits are therefore subject to the Common Technical Specifications (CTS, 2002/364/EC)

Anti-HIV assay kits are validated for use in single donation testing either through CE-marking in the EU or through non-EU regulation. The use of such assays for the testing of pooled plasma for fractionation is a change of the intended use and this application is not covered by the validation undertaken by the test kit manufacturer. The application for testing plasma pools for fractionation therefore has to be accompanied by appropriate validation.

If non-CE marked test kits are used for plasma pool testing purposes, equivalent quality to a CE-marked test kit for individual donation testing should be proven in addition to the validation for pool testing described in this guideline.

The CTS defines minimal requirements for diagnostic sensitivity for assay kits.



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Furthermore, the CTS requires that specificity should be demonstrated in a broad variety of patient samples. However, this approach to validation is not necessarily relevant for plasma pool testing purposes, because those patient samples which may give aberrant responses (e.g., those from patients with autoimmune diseases or having cross-reactive infections) will normally have been excluded by the donor selection process. In addition, non-specific interfering factors will be diluted in the plasma pool.

Plasma pool serology is not capable of detecting all contaminated single donations that may have escaped single donation screening. Anti-HIV is not a defined analyte, but can be described as the sum of reactivity of an individual humoral immune response to a virus subject to high genetic variability. In particular, samples from early infection stages contain low affinity antibodies that show poor dilution kinetics.

Plasma pool serology should therefore not be considered as a test to ensure viral safety, but as a measure to detect serious GMP failures.

This document describes methods to select and validate commercial qualitative immunoassay test kits for assessing contamination of plasma pools with antibodies to HIV 1 and 2 based on the above mentioned documents.

## 2. SELECTION OF THE TEST KIT(S)

Commercial kits used for the analytical procedure are validated by the manufacturer for single donation testing only. Selection of a test kit for plasma pool testing should be based on high dilutional sensitivity. As preliminary selection criterion, relative end point dilution titres of a well-characterised positive sample (e.g. a commercial working Standard) should be compared.

In most cases, the manufacturer's instructions for use of reagents are adequate for the performance of the test procedure on plasma pools.

Any modification of the manufacturer's instructions should be included in the validation of use of a kit for testing plasma pools.

Evaluation criteria of the manufacturer may be adapted to plasma pool testing according to validation data relevant to plasma pools (see 3.1 Specificity and determination of a cut-off limit for pool samples, and 4. Quality assurance).

## 3. VALIDATION

### 3.1 Specificity and determination of a cut-off limit for pool samples

For commercial kits the cut-off value established by the manufacturer is a compromise between sensitivity and specificity based on results from single donation testing. Many test kit manufacturers also define a 'grey-zone' cut-off which will identify samples which give a response above background but below the cut-off. It is recommended that such samples are re-tested as if they were reactive.

On the basis of previous experience in testing plasma pools, the use of a lower cut-off for pool samples should be considered as non-specific factors present in single donations are diluted in a fractionation pool. The use of such a cut-off will increase the analytical sensitivity of assays and facilitate the detection of a single positive donation in a plasma pool. The grey-zone value recommended by the kit manufacturer may be suitable. Alternatively, a limit could be established by considering the signal distribution of negative pools, e.g. as mean response to cut-off ratios of negative pool samples + 3 standard deviations and routinely expressed as % of single donation cut-off. In no case should the pool cut-off be higher than the single donation cut-off.

For all practical applications in the context of this Guideline, if a grey-zone limit is used as the cut-off value for pool samples, this limit is used to identify initially and repeatedly reactive plasma pools (see 5., confirmation strategies).



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### 3.2 Robustness

Robustness of the analytical procedure has to be evaluated, as all methods using biological and biochemical reagents may be subject of considerable batch-to-batch variation of the reagents used and may be influenced by changes in ambient conditions.

#### 3.2.1 Inter-assay and intra-assay

Qualitative immunoassays primarily produce a quantitative signal that is compared to the calculated cut-off in an independent step. Reactive plasma pool samples are likely to give low signals due to high dilution in the pool. Batch-to-batch variability of the test kit reagents (including controls) may have a significant influence on results and should be under control, as is foreseen by the GMP guide (chapter 6, 6.21) and ISO 17025 (4.6.2).

Robustness of the method should be demonstrated for a panel of representative negative pool samples (e.g. routine pool samples which have tested negative by both the manufacturer and an OMCL), and a low positive sample (e.g. the low positive control, see 4.3).

The study should cover:

- Inter-assay variability in 6 independent assays (including variable ambient conditions, equipment if available, preferably using more than one test kit lot if available)
- Intra-assay variability with at least 6 determinations of a low positive control in 1 run

  Intra-assay variability can be expressed as %CV (Relative Standard Deviation, RSD) of the signal of the low positive sample in relation to the cut-off of the individual assay (S/CO, sample to cut-off ratio).

### 3.3 Detection limit

There is no International Standard for antibodies to HIV 1 or 2. As anti-HIV is not a defined analyte, the detection limit (dilutional sensitivity) has to be determined with a representative panel of positive samples reflecting different subtypes and groups taking into consideration the epidemiological situation in the respective regions where the plasma is sourced. Confirmed reactive samples from routine donor screening may be regarded as representative without further characterization, so long as the number of samples in the panel covers the main genotypes.

To facilitate comparability of detection limit data, independent reference material for HIV-1 antibodies should be included in the panel as soon as it becomes available.

The panel is serially diluted in anti-HIV negative pool plasma. The minimum and maximum number of donations in a typical pool should be taken into consideration in the dilution series used to simulate the "best" and "worst"case scenarios. Results are expressed as end point dilution titres.

## 4. QUALITY ASSURANCE

### 4.1 Standard Operating Procedures for plasma pool testing

The test procedures must be described in detail in the form of standard operating procedures (SOPs). These should cover at least the following operations:

- Storage conditions for samples
- Preparation of samples (e.g. freezing/thawing steps, mixing)
- Description of the equipment and the test kit used
- Incubation procedures (including tolerance limits for time and temperature, e.g. according to the test kit manufacturer's specifications/instrument settings)
- Detailed formulae for calculation and interpretation of results
- Validity criteria for the individual assay



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- Retesting procedures
- Reference to confirmation procedures, if applicable

### 4.2 Test kit controls

The test kit manufacturer's controls should always be included in every assay to ensure correct performance of reagents according to the manufacturer's specifications. Validity criteria for modified testing conditions should be defined and documented.

### 4.3 Test kit independent controls

The positive controls in many commercial test kits are highly reactive and therefore do not reflect the low level of reactivity likely to be found in contaminated pool samples. In addition, as for all biological reagents, these controls are subject of batch-to-batch variation. Therefore it is strongly advised to include an independent low positive control (in the dynamic range of the assay, e.g. 2-3 times the single donation cut-off) in every test used for on-going data monitoring.

### 4.4 Proficiency testing

Regular participation in an appropriate proficiency testing scheme which include diluted samples with low reactivity to assess the analytical sensitivity of kits is encouraged.

## 5. CONFIRMATION STRATEGIES

A validated confirmation strategy for initially reactive results should be in place. A pool is considered negative if the initially reactive sample gives a negative result when retested in duplicate. Repeat reactive samples have to be considered positive unless proven otherwise with an adequately validated serological method using different antigens.

A repeat reactive on repeat testing should be confirmed through the use of alternative assays. If immunoblots are used as HIV confirmation tests, great care in the formulation of interpretation criteria is advised, as the highly specific ENV bands are hardly detectable in high dilutions, and some pool samples show unspecific bands at 24 and 40 kDa. Therefore, a positive immunoblot may be used to confirm an initially reactive result. However, a negative immunoblot result should be treated with caution.

As anti-HIV may be present in donors with low or undetectable nucleic acid plasma levels, NAT should not be considered as a confirmation assay as a negative NAT results does not invalidate a positive serological result. On the other hand, positive NAT results do confirm the serological detection of contaminations.

## 6. IMPLEMENTATION OF THIS GUIDELINE

This guideline has been developed to respond to inadequacies in the validation of plasma pool testing for anti-HIV observed during evaluation of dossiers. Marketing Authorisation Holders and Plasma Master File Holders should review the validation of their pool testing methods in the light of this guidance. If the key aspects described in the guideline have already been covered by existing validation, no further validation is needed. If this is not the case, pool testing should be validated in accordance with this guideline and reported in the next annual update of the documentation on the plasma starting material.



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**研究報告の概要**

○中国のHIV/AIDS:数の問題

HIV/AIDSの脅威に対する対応に失敗し、HIV/AIDSの規模を組織的に情報隠蔽した中国は大きな批判にさらされてきた。中国はハイリスク行為に対して法律の施行を中心とした試みに早期に着手したが、後に他国の効果的な施策から得られた教訓によって、エビデンスに基づいた施策を採用したことが報告された。最近発表されたAIDSの予防管理の規制は、中国全土での実施に大きなばらつきがあるとしても、エビデンスに基づく政策の良例である。

HIV感染者の割合は人口の0.05%(65万人)であり他の健康問題が多数あるにも関わらず、注目を集めた理由の一つは、複数の外部団体が行った感染規模の予測が過大な数値だったためである。2002年の国連の委託報告や米国国家情報会議(NIC)は、中国のHIV感染者は約100～200万人で感染爆発の危機が迫っていると推測した。他の研究所からも悲観的な予測値が示された。このような非常に不正確な予測から、様々な問題が生じる。まず、予測されたハイリスク群から一般集団への大規模なウイルスの拡散は起きておらず、根拠のない想定に基づいていたと言える。2番目に、複数の不正確な予測は、専門家でも扇情的な数値を算出の根拠に関係なく受け入れたことを示している。3番目として、過大で不正確な予測が及ぼした影響について問うべきである。中国では、こうした予測に基づいて国内、諸外国、多くの国際民間団体からの多額の援助資金を集めた。この資金は、HIV/AIDSの感染拡大を遅らせたかもしれないが、より必要性の高い領域に援助が届くことを妨げている。

中国でのHIV/AIDS対策はハイリスク地域を中心に行うべきである。

**使用上の注意記載状況・その他参考事項等**

解凍赤血球濃厚液「日赤」
照射解凍赤血球濃厚液「日赤」
解凍赤血球-LR「日赤」
照射解凍赤血球-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 中国のHIV感染者は約100～200万人で感染爆発の危機が迫っていると推測されていたが、実際の感染率は人口の0.05%(65万人)であった。感染規模に関する誤った予測数は中国国内に様々な問題をもたらしたとの報告である。 | 日本赤十字社では、HIVについて20プールNATを含むスクリーニングを行い、陽性血液を排除している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。 |

(十)

Moreover, today's technology drive may soon improve drug-eluting stents and their antithrombotic properties (eg, with use of resorbable drugs, polymers, and perhaps resorbable stents), removing the need for cumbersome long-term antiplatelet therapy and preventing the rare but devastating occurrence of late stent thrombosis.

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PU is a consultant for Cordis and Medtronic, and has received speakers' fees from Cordis, Medtronic, Boston Scientific, Terumo, and Datascope. EDB declares that he has no conflict of interest.

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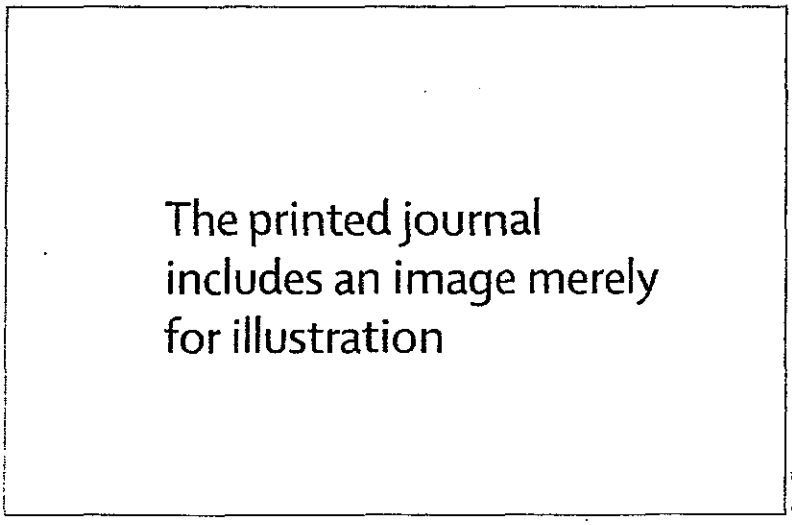
# HIV/AIDS in China: the numbers problem

China has been widely criticised for its failure to respond to the HIV/AIDS threat and for systematic suppression of information about the size of the problem.[1,2] Thus Zunyou Wu and colleagues' report in today's *Lancet* of the way in which China has responded to HIV/AIDS will surprise many.[3] Their thorough review shows how much progress has been made, and how, given the political and cultural context, the Chinese response has evolved in a measured and mainly appropriate way.

Wu and colleagues show how early efforts emphasised enforcement of laws against high-risk behaviour, but that later lessons from effective interventions in other countries (eg, needle-exchange programmes in Australia and condom campaigns for sex workers in Thailand) have led to a more evidence-based approach. The process of policy development might not have been as neat as that presented because of tensions, particularly those between public-health officials and the police and those within public security over the management of illegal drug use and prostitution. However, the recently announced AIDS Prevention and Control Regulations[4] are a good example of evidence-based policy, even if their implementation is highly variable across China.

The most surprising feature of HIV/AIDS in China is how it has attracted such attention and large amounts of external funding, given that the proportion of the

See Editorial page 616
See Comment page 623
See Public Health page 679




Famous Pictures

Patient with AIDS in China

population with HIV/AIDS is only 0·05% and that there are many other more pressing health issues in China. Part of the reason for this attention is because predictions of the size of the epidemic were substantially overestimated by several expert bodies. In 2002, a UN-commissioned report, emotively entitled *China's Titanic peril*,[5] estimated that China had about 1 million cases of HIV, and that it was on the brink of an "explosive HIV/AIDS epidemic...with an imminent risk to widespread dissemination to the general population". The report continued: "a potential HIV/AIDS disaster of unimaginable proportion now lies in wait." A few months later, the US National Intelligence Council estimated that 1–2 million people were living with HIV in China, and predicted 10–15 million cases by 2010.[6] The National Intelligence Council claimed that these figures were more reliable than previous estimates because they did not rely on official sources, which the National Intelligence Council asserted "systematically understate the actual figures", but rather incorporated assessments by academics and non-governmental organisations working in the field.

Other reports at this time were similarly emotive and pessimistic: from the Centre for Strategic and International Studies (Washington, DC, USA), HIV/AIDS was referred to as China's timebomb;[7] and from the American Enterprise Institute as the AIDS typhoon.[8] The latter report emphasised the probable damage to the economy because HIV would spread among young educated urban people. That China had a massive potential HIV problem became received wisdom. However, as Wu and colleagues note, by 2006 the number of people living with HIV/AIDS is estimated to be 650 000—a figure revised downwards by 200 000 from 2005.[9]

Such wildly inaccurate predictions raise several issues. First, how was the figure of 10–15 million cases by 2010 calculated? This estimate assumes substantial spread of the virus from high-risk groups to the general population, yet the few population studies and, in particular, trends from sentinel surveillance of pregnant women in high-risk areas show that such spread has not occurred.[10–12] Therefore, these predictions were made on unfounded assumptions. Second, the inaccurate predictions show how even well-respected groups willingly accept and repeat a number (especially one that is high and sensationalist) irrespective of the assumptions of the underlying calculations, starting a cycle: repeat something often enough and everyone believes it. Third, we should ask what the effects of these high and inaccurate predictions have been. In China, they certainly galvanised activity, attracting large funds from domestic sources (beautifully illustrated by figure 5 in Wu and colleagues' review), international sources (including bilateral donors, notably the UK's Department for International Development and the Australian Government's overseas aid programme, AusAID), and many international non-governmental organisations.

Such funding might have helped slow the spread of the epidemic. However, a disproportionate amount of funding is being given to a health problem for which the disease burden is low, drawing resources away from areas of greater need. For example, China's burden of disease from tobacco use is enormous.[13] If similar resources were devoted to tobacco-control measures, the effect could be huge. The added irony: in a fee-for-service system, individuals can receive free HIV treatment in many areas of China, whereas others have to pay for tuberculosis treatment, drugs for hypertension, or for cataract surgery.

Wu and colleagues call for a scaling-up of HIV/AIDS activities in China. This effort should focus on high-risk areas. For most of China, the prevalence of HIV remains low. Here, the focus of public-health efforts should be on diseases with a higher burden.

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I declare that I have no conflict of interest.

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# HIV-1 in Taiwan

Taiwan is entering a new and dangerous phase of its HIV-1/AIDS epidemic. By the end of 2006, 13702 individuals (including 599 foreigners) had been reported as infected with HIV-1 to the Centers for Disease Control of Taiwan.[1] In 2003, HIV-1 rates in first-time blood donors, military conscripts, and pregnant women were measured at 5·2, 57·0, and 12·0 per 100000, respectively.[2] Data from that year indicated HIV-1 rates of 0·09% for intravenous drug users, 0·2% for female sex workers, 1·9% for patients with sexually transmitted infections, and 6·7% for men who have sex with men in saunas or bath houses.[3] Since then, the number of people living with HIV-1/AIDS in Taiwan has jumped sharply, from an 11% increase in 2003 to a 77% increase in 2004 and a 123% increase in 2005 (figure 1).[1]

However, after the implementation of a harm-reduction programme, a 10% decrease was seen in 2006 (figure 1). The current estimated number of HIV-1/AIDS cases in Taiwan is about 30000, which suggests that the infection rate there could be greater than that in China: 30000 per 23 million (1/767) compared with 650000 per 1·3 billion (1/2000).[2]

A risk-factor analysis of reported cases showed that the proportion of intravenous drug users infected with HIV-1 increased from 1·7% (13/772) in 2002, to 8·1% (70/862) in 2003, to 41·3% (628/1520) in 2004, to 72·4% (2461/3399) in 2005, and dropped to 68·6% (2017/2974) in 2006 (figure 2).[1] The most important risk factor for Taiwanese intravenous drug users is needle-sharing, followed by the sharing of heroin diluents.[3] A molecular epidemiological study showed that more than 95% of intravenous drug users with newly diagnosed HIV-1 in 2004 and 2005 were infected with CRF07_BC, a circulating recombinant form of subtypes B′ and C.[4,5] Previously, several studies suggested that CRF07_BC originated in China's Yunnan province as a mix of subtype B′ from Thailand and subtype C from India. The subtype is believed to have moved to Xinjiang province in China's northwest along a major heroin-trafficking route.[6]

See Editorial page 616
See Comment page 621

Of the 60000–100000 intravenous drug users in Taiwan, 10–15% may be infected with CRF07_BC. If so, they probably represent the largest group of such intravenous drug users in northeast Asia. The circulating recombinant form might have followed a separate drug-trafficking route to Taiwan from Yunnan

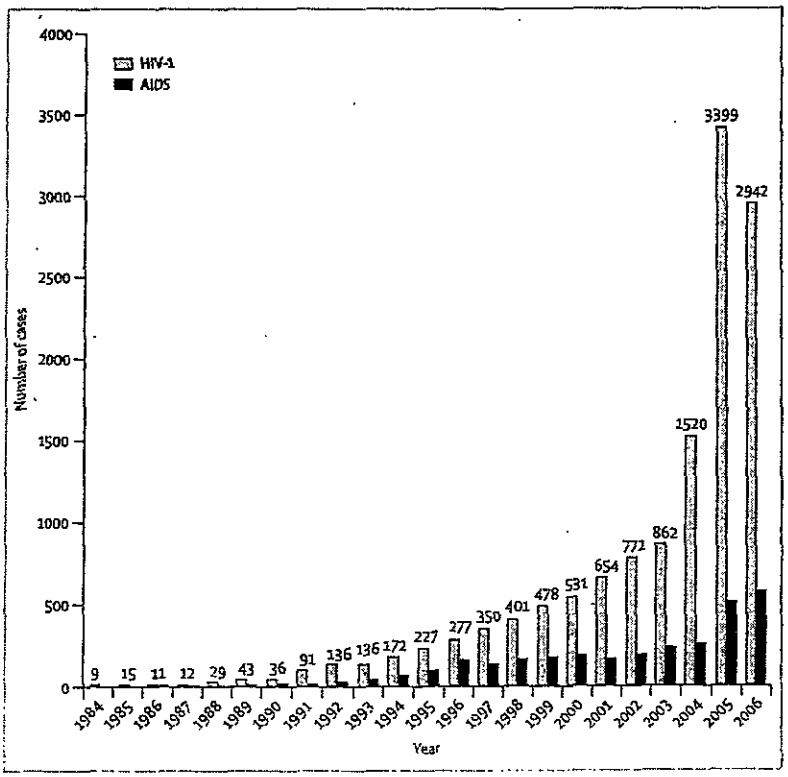


*Figure 1:* Annual numbers of HIV-1 seropositive cases and AIDS patients reported to Taiwan Centers for Disease Control[1]

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| 一般的名称 | ①乾燥抗 HBs 人免疫グロブリン<br>②ポリエチレングリコール処理抗 HBs 人免疫グロブリン | 研究報告の公表状況 | Vox Sanguinis 2007; 92: 113-120 | 公表国<br>韓国 |
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| 販売名（企業名） | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | |

**研究報告の概要**

20 例の血友病患者が、1990 年初頭以降、韓国で製造された血液凝固第 IX 因子の投与を受けてから 1～2 年後にヒト免疫不全ウイルス-1(HIV-1)に感染していると診断された。本研究は血漿ドナーで見出されたウイルスと血友病患者で見出されたウイルスとの間の遺伝子の関連性を調べたものである。感染した血友病患者、韓国製の凝固因子製剤製造に用いられた血漿のドナー、韓国外で感染した血友病患者、および韓国ローカルの対照から得られたウイルスの nef 遺伝子と pol 遺伝子の配列を nestedPCR および直接的な DNA 配列分析で調べた。それらの配列の間の関連性を調べるために系統学的分析を用いた。その結果、血漿ドナーと血友病患者の双方とも、HIV-1 サブタイプ B の韓国 subclade での感染であることが判明した。即ち、これらのデータは、20 例の血友病患者への HIV-1 の伝播が韓国製の凝固因子製剤の静脈内注射を介して起こったことを示している。
更に筆者らは、この報告の中で、当該ロットの血液凝固第 IX 因子製剤を製造した韓国 X 社の製造工程について、以下の問題点があったことを指摘している。
1) 製造法に、New York Blood Center から導入した SD 処理技術を採用していたが、当時レトロウイルスを不活化することが明らかにされていた加熱処理は行なっていなかった。
2) 1991 年半ばに、韓国 X 社の製造施設及び製造工程の査察が行われ、New York Blood Center の専門家の指摘により、以下の工程改善が行われたが、これらの改善は韓国製血液凝固第 IX 因子製剤による HIV 集団感染が起こった後に行われたものであった。
・SD 試薬添加時に二人のサインを求める。
・SD 試薬添加の直前にろ過する。
・SD 試薬添加前にあらかじめ定めたたん白濃度まで希釈する。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 工程管理の不備により、HIV-1 が SD 処理血液凝固第 IX 因子から伝播したとの報告である。<br>弊社で製造工程に SD 処理を導入している製剤には、血液凝固第 IX 因子製剤であるクリスマシン-M のほか血液凝固第 IX 因子製剤であるコンコエイト HT-Wf、トロンビン-ヨシトミ及びフィブリノゲン-HT の 4 製剤がある。<br>これまで、当該 SD 処理 4 製剤が疑われた感染症報告は受けていない。<br>また、当該4製剤のSD処理工程では、SD試薬添加前に薬液の清澄化を目的としてろ過または遠心分離を行っており、工程ではpH、たん白濃度、SD試薬の濃度、温度および処理時間を管理している。またこれらの工程はGMPの下で管理しており、当然のことながら全ての作業は担当者と確認者のダブルチェックのもと行っているために、本報告のような工程管理の不備による感染事故は起こらないと考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**使用上の注意記載状況・その他参考事項等**

代表として静注用ヘブスブリンーIH の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

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ORIGINAL PAPER

# Molecular epidemiologic study of a human immunodeficiency virus 1 outbreak in haemophiliacs B infected through clotting factor 9 after 1990

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Vox Sanguinis

**Background and Objectives** Twenty haemophiliacs were diagnosed as infected with human immunodeficiency virus 1 (HIV-1), 1 to 2 years after exposure to clotting factor 9 manufactured in Korea, beginning in early 1990. This study assessed the genetic relationships between viruses found in plasma donors and haemophiliacs.

**Materials and Methods** Sequencing of the *nef* and *pol* genes of viruses from infected haemophiliacs, plasma donors whose plasma was used in domestic clotting factor manufacture, haemophiliacs infected outside Korea, and local controls were determined by nested polymerase chain reactions and direct DNA sequencing. Phylogenetic analysis was used to investigate the relationships among the sequences.

**Results** Both plasma donors and the haemophiliacs were infected with a subclade of subtype B that is a founder effect lineage in Korea.

**Conclusion** Our data indicate that HIV-1 transmission to 20 haemophiliacs occurred through intravenous injection of Korean-made clotting factor.

**Summary** A clotting factor made in Korea from blood from cash-paid donors infected at least 20 haemophiliacs with HIV-1 subtype B.

Key words: domestic clotting factor 9, haemophiliacs, HIV-1, *nef* gene, phylogenetic analysis, plasma, *pol* gene.

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## Introduction

One consequence of the rapid rate of human immunodeficiency virus 1 (HIV-1) mutation is that phylogenetic analysis of HIV-1 DNA sequences is a powerful tool for identifying closely related viral strains and allowing the inference of transmission between individuals. Many previous studies have used phylogenetic methods to examine suspected or known viral transmission events [1–5]. There are also geographic variations in HIV-1 sequences that may be of scientific use. By comparison to the worldwide sequences, it was found that Korean subtype B (KSB) HIV-1 sequences are quite distinct [6–11], indicating a strong founder effect, with the founding event(s) occurring in the late 1980s.

Prior to 1990, Korean patients suffering from haemophilia B (HP B) were treated with imported clotting factor 9 and other blood products. Domestic clotting factor 9 (DCF), produced by a non-heat inactivation processing, was supplied to almost all Korean HP B beginning in early 1990. Before exposure to DCF through intravenous injection, 18 of the 23 HPs were screened and found to be seronegative for anti-HIV-1

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Y.C. and H.S. participated in designing and performing the research; Y.C., B.T. and J.K. controlled and analysed the data; Y.C., H.S and B.T. wrote the paper; and all authors were involved in preparation and subsequent version of the paper.

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antibodies. Except HP-21 who were diagnosed in 1987, between 1990 and 1994, 22 Korean HPs (19 HP B and 3 HP A) were diagnosed with HIV-1 infection after exposure to DCF. At the time of these diagnoses, there were 122 HP B registered at the Korea Haemophilia Clinic (seropositive rate 15·6%).

In this study, we performed further investigations of the 20 HIV-1-infected HP B and 3 HIV-1-infected HP A patients mentioned above, including a single HP B diagnosed in 1987 prior to receiving DCF, and two HP B who received clotting factors manufactured outside Korea. Considering the very low prevalence of HIV-1 in Korea (251 cases out of a population size of 47 million as of December 1992), the increased prevalence of HIV-1 cases occurring after 1990 within the 122 HP B was very unusual, especially compared to the very low prevalence of seropositives (less than 15 per million) among local blood donors [12]. This large cluster of HIV-1 cases within the haemophilia population prompted the Center for AIDS Research, Korean National Institute of Health, to form an investigation committee to examine this issue.

Before the first anti-HIV-1 antibody testing of HPs registering at the Korea Haemophilia Clinic, 16 of those patients had received transfusions (> 785 units in total, in Table 1). Despite multiple transfusions, they were free of HIV-1 infection until they began to inject DCF, after which time they were rarely transfused (a total 16 units in two HPs). Thus, it seems unlikely that these patients were infected with HIV-1 through transfusion. Interestingly, the only other similar outbreak was seen from 1989 to 1990 in Germany, where 9 of 48 HPs (18·8%) treated with a single contaminated batch of clotting factor 9 were infected with HIV-1 [13].

Here, we investigated whether there is a genetic association between the Korean HIV-1-infected HPs and HIV-1-infected plasma donors who are known to have seroconverted a short time after donating plasma that was used to make DCF.

## Patients and methods

### Twenty-three HIV-1-infected haemophiliacs

HPs 1–20 were diagnosed with HIV-1 infection between 1990 and 1994. HP 21 was infected with HIV-1 by imported factor 9 prior to 1987, and was diagnosed in 1987. Two other HPs (nos 22 and 23) were diagnosed in 1991 and 1994, but they had lived outside Korea for a prolonged period (Table 1). Two other HIV-1-infected Korean HPs were not included from this study, as one was infected with HIV-1 abroad prior to 1987, and the other was infected through mother-to-child transmission. Informed written consent was obtained from all living study participants.

### Cash-paid plasma donors

In the late 1980s, cash-paid plasma collection occurred at domestic plasma centres run by Company X, which manufactures various blood products, including clotting factors. At these centres, four HIV-1-seropositive homosexual donors O, P, Q and R were detected during primary infection (Table 2). The last four plasma units (drawn on 30 November and 8, 20 and 23 December 1989) from donor O were withdrawn, but the prior 79 units had already been used for manufacturing various blood products. Because of the window period between infection and seroconversion, at least four units (drawn on 4, 10, 13 and 16 October 1989) were not safe. Between 3 January 1990 and 26 March 1990, 21 units of plasma were taken from donor P. Three units of plasma (taken on 20 and 23 January and 2 March 1990) from donor P were used to produce DCF according to the manufacturer's report. Two units of plasma were taken from donor Q on 29 January and 6 February 1992. Plasma from donor R was taken nine times between 6 April 1992 and 8 June 1992. Except for last two units, seven units were used for manufacturing blood products. In addition, donors O and R were also infected with hepatitis C virus (HCV) on at least 13 March 1991 and 16 March 1993, respectively.

### Sequencing of the *nef* and *pol* genes

Peripheral blood mononuclear cells (PBMC) from 23 HPs and three donors (O, P and Q) were obtained between 1991 and 2003. To amplify proviral *nef* sequences from the PBMC, nested polymerase chain reactions (PCR) were used as previously described [7]. In cases that were negative for the nested PCR, a second set of nested primers was applied. The primers used were as follows: first PCR, CE21 (forward) and CE22 (reverse); second PCR, primer Nef5'5 and LTR3' [14]. The PCR products were purified and used for direct sequencing. We determined the *nef* gene sequence at least two different times in each HP sample except HP 15. We ruled out PCR contamination by physical separation of each PCR procedure, inclusion of negative controls, BLAST search, and phylogenetic analysis. To amplify partial *pol* sequences from PBMC, a nested PCR was employed as described in our previous studies [11,15].

### *nef* and *pol* sequence data

The GenBank accession numbers for the *nef* sequences from the donors and HPs are AY121450, AY221654, AY121451, AY260770, AY584756–AY584759, AY899339 and AY899340 from donor O; AF063929, AY363309 and AY363310 from donor P; AF063918 from donor Q; AF063923 and AY363311 from HP 1; AF063928 and Z98020 from HP 2; AY221675–AY221715 and AY260771–AY260791 from the other HPs. The local *nef* sequences from non-HPs, which were used for comparison purposes, could be found under the following accession

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Table 1 Summary on 23 HIV-1 infected Korean haemophiliacs

| Haemophiliacs | Age at HIV diagnosis | Deficient factor | Negative for anti-HIV antibody | Dx of HIV infection | Total number of blood transfusion[a] (unit) | Time on the first use of DCF | CD4+ T cell (the earliest) |
|---|---|---|---|---|---|---|---|
| 20 haemophiliacs infected with Korean subclade B of HIV-1 subtype B | | | | | | | |
| 1 | 19 | 9 | April 1990 | 26 November 1990 | Not available (death in 1994) | Early 1990 | 555 in 1991 |
| 2 | 14 | 9 | No data | 21 February 1991 | 33 from February 1986 to March 1989 | May 1990 | 629 in 1991 |
| 3 | 11 | 9 | 21 February 1991 | 1 April 1991 | 3 before 1983 and 93 from 1988 to 1990 | May 1990 | 576 in 1991 |
| 4 | 6 | 8 | No data | 24 August 1991 | 1 in August 1987 | Not available | 826 in 1994 |
| 5 | 11 | 9 | 28 February 1991 | 13 January 1992 | 155 (127 from 1981 to 1988 and 28 in 1989) | February 1991 | 554 in 1992 |
| 6 | 4 | 9 | 9 May 1989/25 February 1991 | 20 January 1992 | 90 from September 1988 to 6 December 1990 (death in 1998) | 14 March 1991 | 771 in 1992 |
| 7 | 7 | 9 | 27 February 1991 | 22 January 1992 | 265 from 1 August 1987 to 23 January 1991 | 1 May 1990 | 804 in 1992 |
| 8 | 19 | 9 | 10 June 1989/9 April 1991 | 15 February 1992 | 16 from June 1989 to September 1990 | April 1991 | 241 in 1992 |
| 9 | 23 | 9 | 27 February 1991 | 25 February 1992 | 5 in Mar 1989 and 3 in 6 April 1991 | 7 March 1991 | 589 in 1993 |
| 10 | 23 | 9 | No data | 26 March 1992 | 56 from March 1990 to September 1990 and 4 in March 1992 | September 1990 | 336 in 1993 |
| 11 | 5 | 9 | 28 February 1990/14 March 1991 | 29 February 1992 | 4 from March 1989 to October 1989 | 16 April 1991 | 966 in 1992 |
| 12 | 29 | 9 | 6 March 1991 | 25 February 1992 | 4 in 1987 and 4 in 1989 | March 1991 | 767 in 1992 |
| 13 | 11 | 9 | 25 February 1991 | 29 February 1992 | 3 in March 1987 | 7 March 1987/11 January 1991 | 446 in 1992 |
| 14 | 35 | 9 | 20 October 1989/22 February 1991 | 19 February 1992 | 24 in October 24 to October 28, 1989 | February 1991 | 420 in 1992 |
| 15 | 16 | 9 | 21 February 1991 | 16 September 1992 | 1 in 1989 | March 1991 | 507 in 1996 |
| 16 | 22 | 9 | 14 March 1991 | 5 December 1992 | 30 from September 1988 to October 1988 | 14 March 1991 | 315 in 1992 |
| 17 | 4 | 9 | 27 February 1991 | 26 February 1993 | 30 to January 1991 | 16 February 1991 | 612 in 1993 |
| 18 | 35 | 9 | 1 July 1991 | 2 March 1993 | Many before February 1988, 2 in March 1988, and 12 in November 1991 | 2 April 1988/10 September 1989 | 173 in 1993 |
| 19 | 13 | 9 | 5 March 1991/2 March 1992 | 26 July 1993 | 0 | 5 March 1991 | 425 in 1996 |
| 20 | 39 | 8 | 27 February 1991 | 4 August 1994 | 0 (use of six vials of DCF instead of factor 8) | 25 February 1991 | 234 in 1994 |
| 3 haemophiliacs infected with non-Korean subclade B of HIV-1 subtype B | | | | | | | |
| 21 | 9 | 9 | No data | 3 August 1987 | Used clotting factors imported from USA | 1990 | 627 in 1987 |
| 22 | 35 | 9 | No data | 27 March 1991 | 4 in January 1987, lived in USA in 1988 | 1990 | 527 in 1991 |
| 23 | 10 | 8 | No data | 13 October 1994 | Lived in Iran for 10 years since birth | October 1994 | 561 in 1996 |

[a]Blood transfusion includes fresh frozen plasma, cryoprecipitates, and so on. Haemophiliacs 13 and 18 were also exposed to DCF which was manufactured in 1987. Dx, diagnosis; DCF, domestic clotting factor 9.

Table 2 Summary on the four HIV-1 infected cash-paid plasma donors and domestic clotting factor (DCF)

| Plasma donor | Age at HIV diagnosis | Period of plasma donation at Company X | Unit of plasma donation | Last negative for anti-HIV antibody | Diagnosis of HIV infection |
|---|---|---|---|---|---|
| O | 27 | 5 January 1988 to 23 December 1989 | 83 | 16 October 1989 | 30 November 1989 |
| P | 30 | 3 January 1990 to 26 March 1990 | 19 | 15 March 1990 | 30 March 1990 |
| DCF has been manufactured from late 1989 in Company X | | | | | |
| Q | 32 | 29 January 1992 to 6 February 1992 | 2 | 29 January 1992 | 6 February 1992 |
| R | 22 | 6 April 1992 to 8 June 1992 | 9 | 30 May 1992 | 3 June 1992 |

numbers: AF462701–AF462767, AY121449–AY121471, AY363309–AY363369 and AY584754–AY584808.

The GenBank accession numbers for the 532 *pol* sequences from the donors, HPs and local sequences from non-HPs are as follows: AY585687 and AF407364 from donor O, AY347694 from donor P, AY347690 from HP 1, AY166460–AY166503, AY219009–AY219031, AY347683–AY347709, AY392099–AY392125 and AY731184–AY731229 for other HPs and local controls.

### Phylogenetic analysis

The sequences described in the current study were aligned to the HIV-1 subtype reference set from the HIV Sequence Database (http://hiv-web.lanl.gov/content/hiv-db/Subtype_REF/align.html) and phylogenetic trees were built using the PHYLIP DNADIST (F84 model, Ts : Tv 1·7) and NEIGHBOUR programs. Trees built using the maximum parsimony method in PAUP produced identical trees with regard to which sequences fell within KSB within HIV-1 subtype B.

## Results

### Characteristics of haemophiliacs

Seventeen of 20 HPs (excluding nos 2, 4 and 10) screened negative for HIV-1 antibody just before they were administered (Table 1). The test was mainly performed by the Korea Haemophilia Clinic using an internationally marketed ELISA kit manufactured by Company X. In addition, four of the HPs (nos 6, 8, 11 and 14) tested negative for HIV-1 antibody at other university hospitals 1–2 years before using DCF. This group was mainly treated with imported factors prior to the inception of domestic DCF production (officially 12 December 1989, although 154 vials were produced in 1986).

### Molecular epidemiologic data from the *nef* gene

Phylogenetic analysis revealed that 20 of the HPs (nos 1–20) and the three plasma donors (O, P and Q) were infected with KSB HIV-1, whereas the three HPs (nos 21–23) infected abroad were infected with non-KSB HIV-1. HPs 1–4 were the first cases among 20 HPs infected with the KSB (Fig. 1). As expected from the time period between seroconversion and sampling for DNA sequencing for this study, intrapatient DNA sequence identity among the HPs who had progressed to AIDS (HPs 3, 5, 16 and 21) was less than 95%. In contrast, in patients whose CD4+ T cell count maintained a steady state, such as HPs 7 (98·7%), 8 (95·1%), 13 (97·8%), 14 (96·6%), 19 (96·4%) and 22 (96·2%), sequence identities were > 95% after 7–9 years of infection.

### Molecular epidemiologic data from the *pol* gene

We determined *pol* sequences from donor O (957 bp: AY585687), donor P (543 bp: AY347694, derived from September 1991), and 292 local control sequences from 107 local patients not known to have sold plasma for DCF production. In a phylogenetic tree including 23 domestic *pol* sequences (> 957 bp), sequences from eight HPs strongly clustered around those of donor O without the embedding of other local sequences (Fig. 2), and sequences from 10 HPs also strongly clustered. Although we could not include *pol* sequences from donor P in this phylogenetic tree or for comparison of sequence identities due to small fragment size of 543 bp, sequences from 10 HPs were clustered without an embedding of other local sequences in the lower cluster.

In regard to the frequency of the specific amino acid reverse transcriptase (RT) codons in KSB sequences, I135V, I202V, and R211K were detected in four, six and six of 45 local control patients (including two cash-paid plasma donors), respectively (Table 3). In contrast, the 20 HPs showed significantly higher frequencies of these three codons, in a manner consistent with their occurrence in the two cash-paid plasma donors. Specifically, two RT sequences from donor O showed I202V but not I135V and R211K, whereas those of donor P showed I135V (sequences after codon 190 were not determined). The frequencies of I135V, I202V and R211K (9, 9 and 12, respectively, based on the earliest sequences from each patient) were significantly higher in the 20 HPs than in the 45 local control patients ($P < 0·01$ by Student's *t*-test for all three codons) (Table 3). This finding also supports the epidemiological linkage for the transmission of HIV-1 from two plasma donors to at least 17 HPs.

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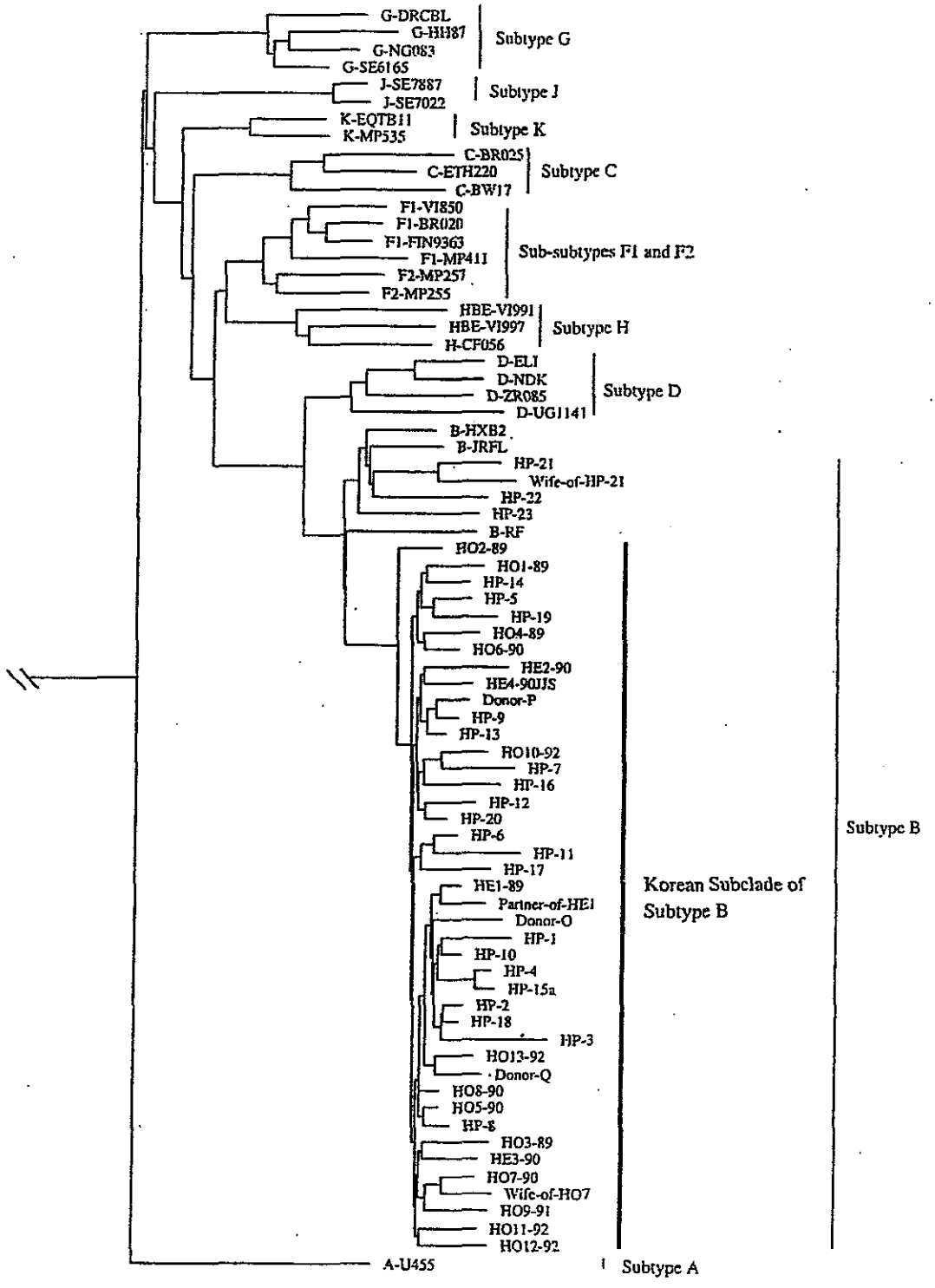


Fig. 1 Phylogenetic tree of *nef* sequences of 23 haemophiliacs (HP), plasma donors (O, P and Q) and 19 database *nef* sequences that showed the highest BLAST score with donors O and P and the HIV Database subtype reference set (HO means sequences obtained from homosexuals and HE, heterosexuals). The sequences described in the current study were aligned to the HIV-1 subtype reference set from the HIV Sequence Database (http://www.hiv.lanl.gov/content/hiv-db/SUBTYPE_REF/align.html) and sequences scoring highly in BLAST, and a phylogenetic tree was built using the PHYLIP DNADIST (F84 model, Ts : Tv 1·7) and NEIGHBOUR programs.

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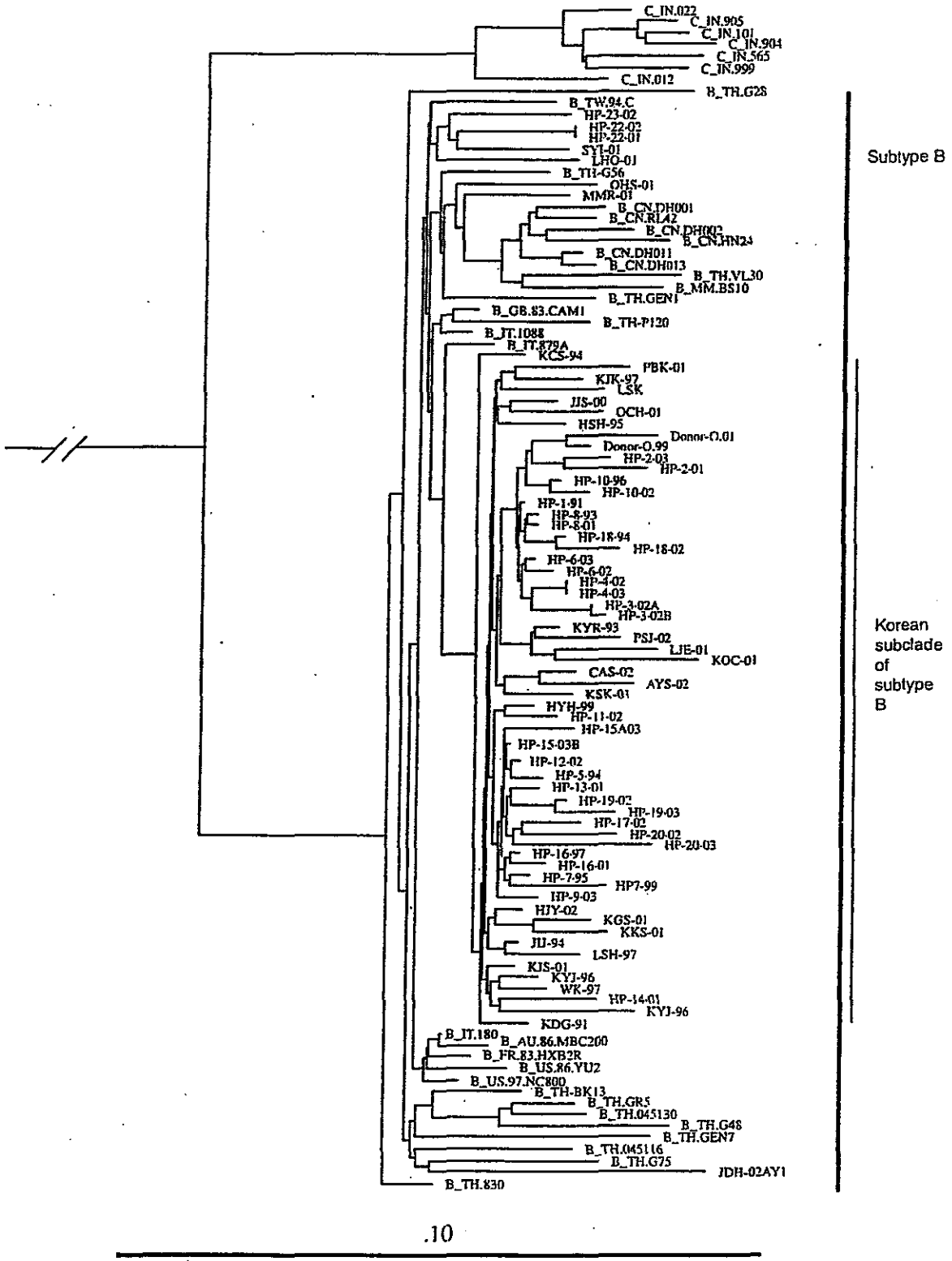


Fig. 2 *Phylogenetic tree of pol sequences of 23 haemophiliacs (HP), plasma donor O and 23 local sequences. Twenty-three local pol sequences = 957 bp were selected by the highest BLAST score with the two plasma donors. We did not include the pol sequences from donor P because of the shorter length (543 bp) sequenced from this sample. There are two strong clusterings with 18 HPs; sequences from eight HPs strongly clustered around those of donor O and sequences from 10 HPs also strongly clustered.*

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Table 3 Frequency of specific amino acids in reverse transcriptase (RT) of haemophiliacs is statistically significantly higher than those in 45 local HIV-1 with Korean subtype B

| | Reverse transcriptase codons (consensus) | | | |
|---|---|---|---|---|
| Haemophiliacs | Codon 135 Ile | Codon 202 Ile | Codon 211 Arg | GenBank no. |
| Donor P | Val | No data | No data | AY347694 |
| 5 | $Val_2$ | ·/$Val_1$ | $Lys_2$ | AF273194 |
| 12 | $Val_2$ | ·$_2$ | $Lys_2$ | AY219017 |
| 13 | $Val_1$ | ·/$Val_1$ | ·/$Lys_1$ | AY166480 |
| 15 | $Val_3$ | ·$_3$ | $Lys_3$ | AY347701 |
| 17 | $Val_3$ | ·$_3$ | $Lys_3$ | AY219021 |
| 19 | $Val_3$ | ·$_3$ | $Lys_3$ | AY219023 |
| 20 | $Val_3$ | ·$_3$ | $Lys_3$ | AY219024 |
| 14 | $Val_2$/. | ·$_3$ | $Lys_3$ | AF273196 |
| 16 | Val/. | ·$_2$ | $Lys_2$ | AY392120 |
| 7 | ·$_7$ | ·$_7$ | $Lys_7$ | AF448137 |
| 11 | ·$_2$ | ·$_2$ | $Lys_2$ | AY219020 |
| 9 | $Leu_4$ | ·$_4$ | $Lys_4$ | AY347699 |
| Donor O | ·$_2$ | $Val_2$ | ·$_2$ | AF273180 |
| 1 | ·$_1$ | $Val_1$ | ·$_1$ | AY347699 |
| 2 | ·$_4$ | $Val_4$ | ·$_4$ | AF448135 |
| 3 | ·$_3$ | ·$_1$/$Val_2$ | ·$_2$/$Lys_1$ | AF273186 |
| 4 | ·$_2$ | $Val_2$ | ·$_2$ | AY219015 |
| 6 | ·$_2$ | $Val_2$ | ·$_2$ | AY219016 |
| 8 | ·$_3$ | $Val_3$ | ·$_3$ | AF481437 |
| 10 | ·$_2$ | $Val_3$ | ·$_3$ | AY219012 |
| 18 | ·$_9$ | $Val_{17}$ | ·$_{17}$ | AY166489 |

Number in subscript means times sequenced in each patient. A dot '·' indicates identity to the consensus for this codon.

## Discussion

In this study, we found that 20 of 23 Korean HPs were infected with KSB HIV-1. Our investigation of molecular epidemiology rules out foreign imported factor 9 as the cause of infection in the 20 HPs and indicates that DCF is the most probable causative factor for this HIV-1 outbreak. This is further supported by medical record evidence that shows that all 20 HIV-1-infected HP with KSB were exposed to the same DCF. This was manufactured from plasma including donations of plasma from donors O and P collected a relatively short time prior to their documented seroconversion dates.

Eighteen of the 23 HIV-1-infected HPs had tested HIV-1 seronegative at the beginning of DCF therapy, but became seropositive within 2 years of using DCF. Second, more than 50% of the HPs were sexually inactive at the time of diagnosis. Third, in a case–control study taken as one arm, there were statistical associations between six lots of DCF and HIV-1 infection in HPs described in a report from an investigative committee (April 2004). Fourth, the prevalence of HIV-1 infection in the general population was very low at the time of the outbreak. A similar overseas study [6] also showed that only a portion of HPs exposed to contaminated clotting factor 9 developed HIV-1 infection.

Despite the 7–10 years time lag between the outbreak in 1991–1992 and our sampling in 1998–2002, our data show that the sequences from 20 HPs were most closely related with those from two plasma donors. Taken together, both the epidemiological data and this molecular data support the conclusion that HIV-1 transmission to most of the infected Korean HPs occurred from a common source, which is the intravenous injection of DCF, rather than transfusions or imported clotting factors.

DNA sequences and sequence analysis can indicate that virus transmission is likely to have occurred between one person and another. However, other relevant epidemiological information related to such cases must always be used in concert with the molecular epidemiology data. In this case, it is the combination of records providing the dates of cash-paid plasma donations, plus records providing the seroconversion dates of the donors and HPs, together with the molecular data establish the near certainty that the HPs were infected via the clotting factor. Even taken together, these data cannot prove that any one or more of these donors was the cause of the HP infections. It is possible that each HP received some virus from both donors, and also possible that donor Q or other, as yet unidentified donors also contributed to this problem. The only strong linkage is between the DCF product and this group of HPs. The individual cases, such as some HPs having sequences slightly closer to sequences from donor O and others having sequences slightly closer to donor P, are not highly significant. We do not have enough information about pooling of plasma donations and production sizes of batches of DCF to know if it is likely or possible that each of the HPs would have been exposed to virus from only one donor or to a combination of viruses from more than one donor.

Regarding the DCF manufacturing technique, Company X employed a solvent/detergent (tri-*n*-butylphosphate/Tween 80) technique adapted from the New York Blood Center (NYBC), with no heat treatment. After reporting a series of HIV-1 cases in US HPs, several improvements were implemented according to an investigator from NYBC. During an in-house audit of the Company X's manufacturing facility and processing as part of the activity of the investigation committee, a specialist from the NYBC noted that 'several improvements in processing were instituted in mid-1991, including use of a double signature addition, filtration just prior to solvent/detergent addition, and dilution to preset protein concentration' (Horowitz B: solvent/detergent usage by the Company X-summary of technology transfer and 2-day site visit. *Report* signed on 1 June 1993). This mid-1991 time point was after the detection of the first cases of infection in this cohort (in HPs 1, 2, 3 and 4). Still no heat

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treatment that has been shown to inactivate retroviruses [16,17] was used.

Korean subtype B sequences were first detected in homosexual Korean HIV-1 patients diagnosed in 1989 [7–11]. Our previous studies [7–11] showed that the KSB sequences represent a subclade of global subtype B, indicating a founder effect. All KSB sequences were found among domestic-residing homosexuals who did not have sexual contact with foreigners. In contrast, Korean patients infected with non-KSB HIV-1 had sexual contact with US army soldiers in Korea in the late 1980s or had visited the USA, whereas overseas sailors who had visited foreign countries and their spouses showed various subtypes according to their epidemiological history [7–11]. A total of 128 HIV-1-infected patients (1 in 1985, 4 in 1986, 9 in 1987, 22 in 1988, 37 in 1989 and 54 in 1990) were diagnosed in Korea before 1991. Based on epidemiological data, 37 of those were presumed to be infected with KSB and 91 with non-KSB including 59 overseas sailors, five of their wives, and 10 prostitutes who had worked next to US military camps in Korea.

In conclusion, the *nef* and *pol* sequences from donors O and P showed higher DNA sequence identities with those from the tested HPs than with local sequences from Korean seropositive individuals including homosexuals diagnosed with KSB HIV-1 infection before 1991. These data coincide with the clinical and medical records, and together indicate that HIV-1 transmission to 20 HPs occurred through IV injection of DCF rather than transfusions or imported clotting factors.

## Acknowledgements

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| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | Science 2006; 313: 1781-1784 | 公表国<br>ドイツ | | |
| 販売名<br>(企業名) | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④コンコエイト－HT（ベネシス）<br>⑤クリスマシン－M（ベネシス） | | | | | |

**研究報告の概要**

タンパク質の凝集はアルツハイマー病の発病機序として確立されてはいるが、そのプロセスが in vivo でどのように開始されるのかという点についてはほとんど明らかとなっていない。アルツハイマー病患者、またはβ-アミロイド前駆体タンパク質(APP)を発現しているトランスジェニックマウスから得たアミロイド-β(Aβ)を含んでいる脳抽出物の希釈したものを APP トランスジェニックマウスの大脳内に注射すると、時間と濃度に依存した大脳内のβ-アミロイドーシスとそれに伴う病変を誘導した。APP23 宿主マウスを Beta-1 抗体で受動免疫させると誘導される病変の進行が阻害された。さらに、70％蟻酸での 1 時間処理によりアミロイド誘導活性を完全に消失させたが、95℃、5 分間の熱処理はアミロイド誘導を消失させなかった。
脳抽出物のシーディング(seeding)活性は、Aβ免疫除去、タンパク変性、またはAβを宿主に免疫することによって、低下または消失する。外因性に誘導したアミロイドーシスの表現型は、宿主と誘導因子のソースの双方に依存して変わり、そのことは、プリオン系統を思わせるような、生物活性の異なる多型性Aβ系統の存在を示唆している。プリオン病はさまざまな伝播効率で種々の経路を介して野生型の宿主へ伝播することができ、プリオンの取り込みと分布に全身性の細胞機作が関与しているが、現在のところ、β-アミロイドーシス(特に AD)が、プリオン病と同じ意味で伝播性であるという証拠はない。しかし、異常Aβアセンブリーの誘因と増殖に関与する機作を理解することによって、特発性アルツハイマー病の起源を解明しうることとなろう。

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wf の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-1 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している.更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アルブミンを精製し、アルブミン濃度 5w/v％に調整した製剤であり、ウイルス不活化を目的として、製造工程において 60℃、10 時間の液状加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| アルツハイマー病患者のアミロイド-βが異常プリオン蛋白質と同様の感染性を持っている可能性が示唆されるとの報告である。<br>アルツハイマー病患者由来のアミロイド-βの感染性を示唆する情報は、トランスジェニックマウスへの脳内接種による感染実験のみであり、アルツハイマー病が実際に感染･伝播したとの事例は報告されていない。そのため、アルツハイマー病患者のアミロイド-βが異常プリオン蛋白質と同様の感染性を持っていると結論付けるには情報が乏しいと考える。 | アルツハイマー病に関連する情報については、今後も注視することとする。 |

16

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**Supporting Online Material**
www.sciencemag.org/cgi/content/full/313/5794/1775/DC1
Materials and Methods
Figs. S1 to S4
References and Notes

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# Exogenous Induction of Cerebral β-Amyloidogenesis Is Governed by Agent and Host

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Protein aggregation is an established pathogenic mechanism in Alzheimer's disease, but little is known about the initiation of this process in vivo. Intracerebral injection of dilute, amyloid-β (Aβ)–containing brain extracts from humans with Alzheimer's disease or β-amyloid precursor protein (APP) transgenic mice induced cerebral β-amyloidosis and associated pathology in APP transgenic mice in a time- and concentration-dependent manner. The seeding activity of brain extracts was reduced or abolished by Aβ immunodepletion, protein denaturation, or by Aβ immunization of the host. The phenotype of the exogenously induced amyloidosis depended on both the host and the source of the agent, suggesting the existence of polymorphic Aβ strains with varying biological activities reminiscent of prion strains.

The accumulation of misfolded proteins is a common feature of several neurodegenerative disorders. In Alzheimer's disease (AD), the multimerization of the Aβ peptide is an early and central process in the pathogenic cascade (*1*–*3*), but little is known about the mechanisms that govern the initiation of Aβ aggregation and deposition in vivo. Ordered protein aggregation in vitro is a function of protein concentration and time and follows a crystallization-like polymerization mechanism that can be rapidly initiated by introducing an exogenous seed (*4*). In vivo, seeded aggregation of Aβ is seen after injecting AD brain extracts into the brains of non-human primates (*5*) or APP-transgenic mice (*6*), reminiscent of the conformational conversion mechanism of prion infectivity (*7*–*9*).

We injected 10% (w/v) extracts of brain homogenates from autopsied AD patients (AD extract) or from aged, β-amyloid–laden APP23 transgenic mice (*10*) (APP23 Tg extract) into the hippocampus of young male APP23 mice. Four months later, the host mice were analyzed (*11*). Both AD extract and APP23 Tg extract induced robust deposition of Aβ in the hippocampus (Fig. 1, A and B). Intracerebral injection of tissue extract from an aged control patient induced only minimal Aβ deposits, consistent with the low Aβ load in the donor (Fig. 1C; fig S1). No seeded Aβ deposits were found after control injections of brain extract from an aged, wild-type mouse or of phosphate-buffered saline (PBS) (Fig. 1, D and E). Infusion of APP23 Tg extract into wild-type mice did not induce Aβ deposition; thus, the observed Aβ deposits did not simply represent the

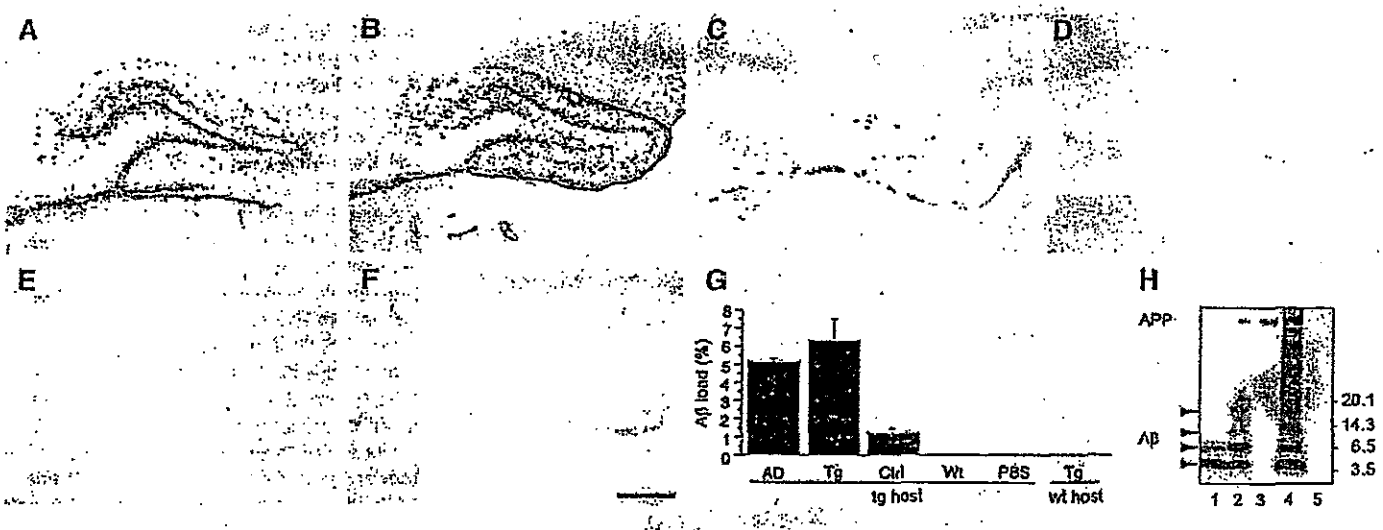


Fig. 1. Brain extract (10%) was injected into the hippocampus of 5-month-old male APP23 hosts (A to E) and nontransgenic littermates (F). Mice were analyzed 4 months later. Injection of AD extract (A) and APP23 Tg brain extract (B) induced numerous Aβ-immunoreactive deposits. Few or no Aβ deposits were detected after injections of brain extract from an aged (95 years) control (Ctrl) patient (C) or wild-type (Wt) mouse (D). No Aβ deposits were observed after PBS injections (E) or when Tg extract was injected into wild-type mice (F). Stereological quantification of Aβ load by immunohistochemistry (G) confirmed significant amyloid induction by AD and Tg brain extracts compared to Ctrl and Wt extracts ($n = 5$ mice per group; mean ± SEM, $P < 0.001$). (H) Tris-Tricine SDS–polyacrylamide gel electrophoresis (PAGE) followed by immunoblotting with a human Aβ-specific antibody. Lane 1: synthetic Aβ1-40 + Aβ1-42 (1 ng/μl each); lane 2: AD brain extract; lane 3: Ctrl brain extract; lane 4: APP23 Tg brain extract; and lane 5: Wt brain extract. For each extract, 1 μl was loaded. Arrowheads indicate monomeric, dimeric, trimeric, and tetrameric Aβ. Aβ concentration in the control patient extract was below the detection level. When less dilute samples were used, Aβ was detected in the Ctrl extract, consistent with the sparse amyloid plaques in this patient (fig. S1) and the modest seeding activity of the Ctrl extract. Scale bar, 350 μm.

injected Aβ-containing material (Fig. 1F), and imply that host factors are critical for in vivo seeding. No seeded Aβ deposits were observed when APP23 Tg extract from a young, 2-month-old, predepositing mouse was injected into APP23 hosts. The concentrations of Aβ in the AD and APP23 Tg extracts were estimated to be 1 to 10 ng/μl. In both AD extract and APP23 Tg extract, Aβ monomers, oligomers, and larger multimeric species were present (Fig. 1H).

The localization and biochemical nature of the induced Aβ lesions were markedly similar to those seen in normal, aged APP23 transgenic mice (*10*, *12*). Induced Aβ deposits were found primarily in the injected hippocampus, but some were also observed in the dorsal lateral geniculate nucleus, corpus callosum, and entorhinal cortex, and in the vasculature of the thalamus and pia mater (fig. S2). Most induced Aβ deposits were diffuse, although some congophilic amyloid plaques were present and surrounded by activated microglia, astrocytes, and dystrophic neurites (fig. S2). Aβ in micropunches taken from the hippocampus of injected APP23 mice was mainly parechymal and consisted of Aβ1-40 and Aβ1-42, whereas the amyloid in the thalamic micropunches was mostly vascular and consisted predominantly of Aβ1-40 (fig. S3).

The exogenous induction of Aβ deposition in vivo also is time and concentration dependent (fig. S4). Immunoreactive Aβ deposits first appeared 2 months after injection and thereafter increased significantly with time. APP23 extract that was further diluted to 0.5% produced a pattern of deposition in the host similar to that seen with the 10% extract, but with much less potency (fig. S4).

The phenotype of exogenously induced β-amyloid deposits is dependent on both the agent and the host. We injected brain extract from aged APP23 mice into APP–presenilin-1 (PS1) transgenic hosts, and vice versa (Fig. 2). Amyloid deposition in the hippocampus of male APP23 mice normally begins at 9 to 10 months of age, whereas APPPS1 mice develop Aβ deposition in the hippocampus at 3 to 4 months of age (*10*, *13*). Total Aβ concentration was similar in the two extracts (Fig. 2I), but in APPPS1 extracts, the highly amyloidogenic Aβ1-42 was several times as abundant as Aβ1-40, whereas in extracts from

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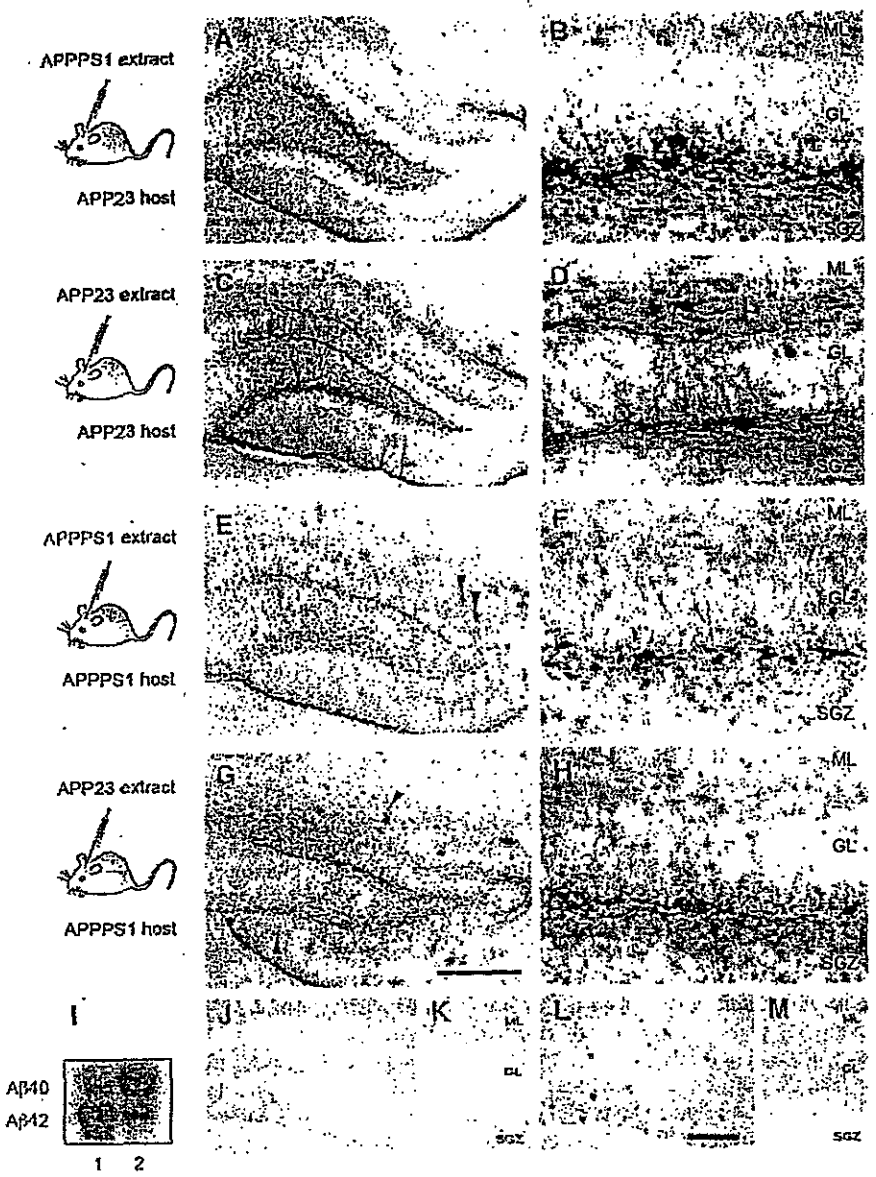


**Fig. 2.** Brain extract (10%) from an aged APPPS1 mouse or an aged APP23 mouse was injected intrahippocampally into either 5-month-old male APP23 or 2-month-old male APPPS1 hosts. Brains were immunohistochemically analyzed for Aβ 3 months later. The panels to the left (A, C, E, and G) show overviews of the hippocampus, and the panels to the right (B, D, F, and H) show corresponding higher magnification images of the upper blade of the dentate gyrus (ML, molecular layer; GL, granular cell layer; SGZ, subgranular cell layer). The coarse, punctate pattern of Aβ staining in the APP23 host is apparent, particularly in the SGZ, after injection of the APPPS1 extract (A and B). Injection of APP23 extract induced more filamentous and diffuse Aβ in the ML (C and D). When APPPS1 extract was injected into the APPPS1 host, the coarse, punctate pattern of Aβ induction was even more distinct (E and F), whereas the APP23 extract in APPPS1 hosts induced Aβ lesions intermediate in appearance to the coarse and filamentous types (G and H). At 5 months of age, APPPS1 hosts had developed some Aβ deposits endogenously, predominantly in the ML [arrowheads in (E) and (G)]. For comparison, an 8-month-old noninjected male APP23 mouse (J and K) and a 5-month-old noninjected male APPPS1 mouse (L and M) are shown. Scale bar, 350 μm. (I) Urea-based SDS-PAGE immunoblot analysis with an antibody specific to human Aβ. Lane 1: APPPS1 extract; lane 2: APP23 extract. Aβ1-42 was the major Aβ species in APPPS1 mice, whereas Aβ1-40 predominated in the APP23 extract. Total Aβ was comparable in the two extracts [APPPS1: total Aβ, 14.7 ng/μl (Aβ40: 4.6 ng/μl; Aβ42: 10.1 ng/μl); and APP23: total Aβ, 11.7 ng/μl (Aβ40: 8.1 ng/μl; Aβ42: 3.6 ng/μl)].

APP23 mice, more Aβ1-40 was present. The amyloid-inducing activity of APP23 and APPPS1 extracts was quantitatively similar 3 months after infusion, but injection of APPPS1 extract into APP23 hosts consistently induced a coarse pattern of compact, punctate Aβ deposition that was mainly confined to the subgranular layer of the hippocampus, whereas the APP23 extract injected into APP23 hosts yielded primarily diffuse and filamentous lesions, with substantial diffuse Aβ in the molecular layer (Fig. 2, A to D). When the same extracts were injected into the APPPS1 host, lesions induced by the APPPS1 extract were even more coarse and punctate, whereas those induced by the APP23 extract were a mixture of filamentous and compact types (Fig. 2, E to H). In mice analyzed 1 month after the infusion of the extract, appreciable coarse Aβ induction was already apparent in APPPS1 hosts but not in APP23 hosts.

To establish whether Aβ is a prerequisite for the amyloid-inducing activity of the extract, APP23 Tg extracts were either Aβ-immunodepleted (Fig. 3, A to C) or mixed with the Aβ-specific antibody (anti-Aβ) Beta-1 (Fig. 3, D to F) and injected into young APP23 hosts. Immunodepletion completely prevented the amyloid-inducing activity of the extract, whereas the Beta-1–containing extract attenuated amyloid induction by >60% compared to a control antibody.

Passive immunization of APP23 host mice with Beta-1 antibodies inhibited the development of induced lesions. Administration of the antibodies commenced 4 weeks after the intracerebral injection of APP23 Tg extract in order not to interfere with the initial seeding process. Serum anti-Aβ titers of 1:2400 to 1:8000 were maintained until mice were killed 4 months after the extract infusion. Amyloid induction was almost completely inhibited in immunized mice compared to those injected with control antibody

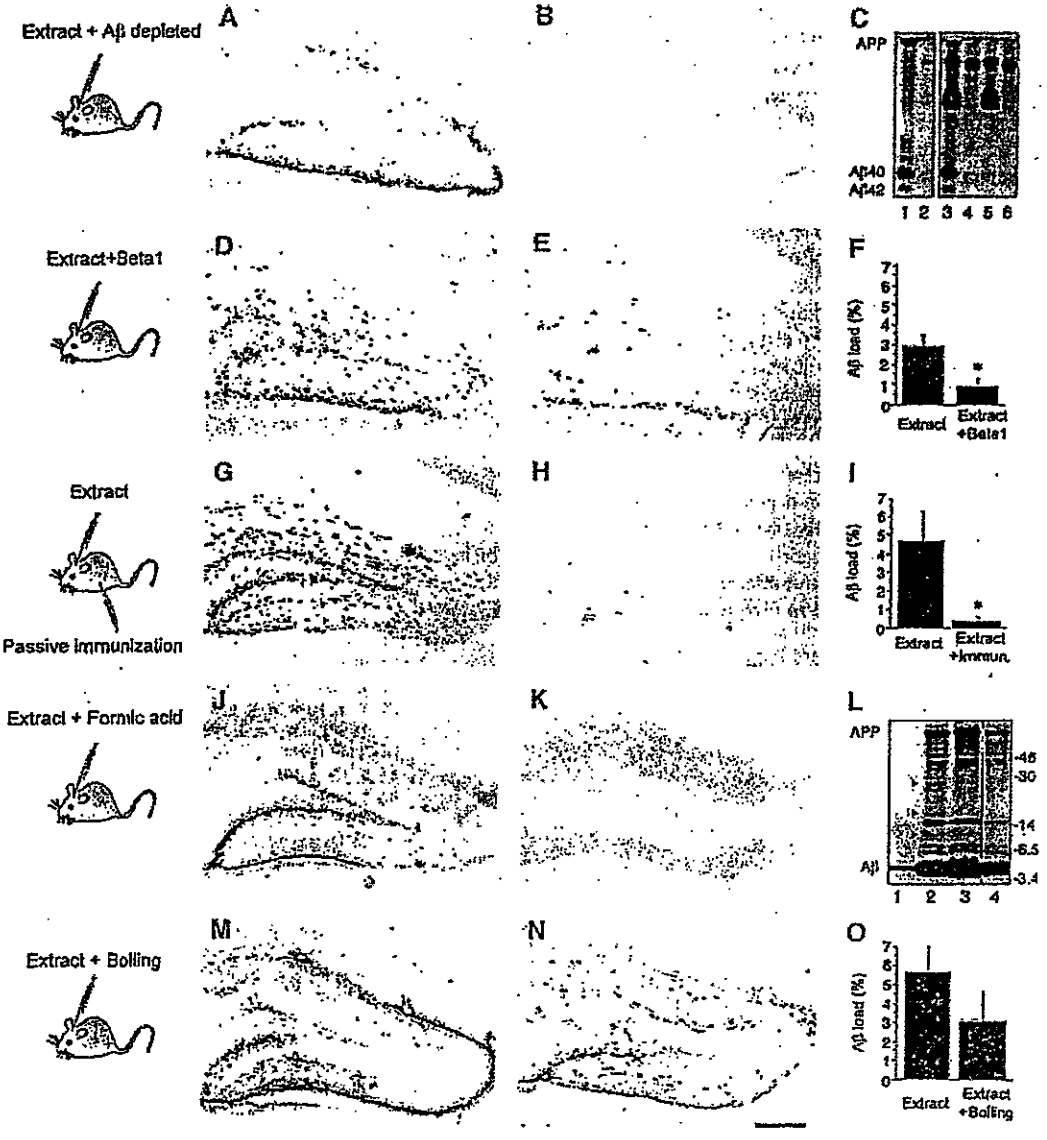


**Fig. 3.** (A to C) Immunodepletion: APP23 Tg brain (0.5%) extract (A) or the same extract Aβ-immunodepleted (B) was injected intrahippocampally into 3-month-old APP23 mice that were then analyzed 3 months later. Immunodepletion completely eliminated the amyloid-inducing activity of the extract. (C) Urea-based SDS-PAGE followed by immunoblotting with human Aβ-specific antibody. Lane 1: intact APP23 Tg extract; lane 2: immunodepleted extract (the absence of detectable Aβ and APP fragments is apparent); lanes 3 to 6: eluted pellet fractions after the first, second, third, and fourth depletion steps. (D to F) Antibody blocking: APP23 or AD brain extract (10%) was mixed with either a control antibody or with anti-Aβ Beta-1 and injected into 6-month-old male APP23 mice. Four months after injection, amyloid induction was significantly lower in the Beta-1–injected mice (E) than in the control mice (D), as confirmed by stereological quantification of immunoreactive Aβ (F) ($n = 5$ mice per group; $P < 0.05$). (G to I) Immunization: APP23 or AD brain extract (10%) was injected intrahippocampally into 6-month-old male APP23 mice, followed by weekly peripheral injections of Beta-1 antibody (H) or control antibody (G), starting 1 month after the brain-extract injections. Four months later, immunohistochemical (G and H) and stereological (I) analysis revealed a >90% inhibition of amyloid induction by passive immunization ($n = 5$ mice per group; $P < 0.001$). (J to L) Formic acid treatment: APP23 Tg extract (10%) was treated with formic acid and injected into the hippocampus of 3-month-old APP23 mice that were analyzed 3 months later. (K) Formic acid completely nullified the amyloid-inducing activity of the extract. (J) PBS-treated control extract. (L) Bicine-Tris SDS-PAGE (without urea) followed by immunoblot analysis with human Aβ-specific antibodies. Lane 1: synthetic Aβ (2 ng/μl); lane 2: PBS-treated control APP23 extract (10 μl); and lanes 3 and 4: formic acid–treated APP23 extract (20 μl in lane 3 and 10 μl in lane 4). The oligomeric bands are absent or greatly reduced (arrowheads) in the formic acid–treated extract, even at twice the concentration of the control extract. (M to O) Heating: APP23 extract (10%) was heated to 95°C (N) before injection into the hippocampus of 3-month-old APP23 mice. Analysis 3 months later showed that heating reduced (45%) but did not eliminate Aβ seeding ($n = 3$ mice per group; $P > 0.05$). Scale bar, 350 μm.

(Fig. 3, G to I). An active immunization protocol revealed similar inhibition (fig. S5).

The amyloid-inducing activity of the extract was disrupted by formic acid denaturation. APP23 Tg extracts were treated for 1 hour with 70% formic acid, followed by dialysis, or heated to 95°C for 5 min, cooled, and injected into young APP23 mice. Formic acid treatment completely abolished the amyloid-inducing activity of the extract, whereas heating reduced, but did not eliminate, amyloid induction (Fig. 3, J to O).

Preparations of soluble or fibrillar synthetic Aβ40, Aβ42, or a mixture of both, in amounts similar to those of Aβ in APP23 Tg brain extracts, failed to induce detectable Aβ deposition (Fig. 4). Injection of synthetic Aβ at concentrations 100 to 1000 times that of Aβ in APP23 Tg brain extracts resulted in amorphous masses of material near the injection site that consisted primarily of the injected material. Few newly generated aggregates were present, as confirmed by infusion of biotinylated Aβ1-42 (fig. S6). Synthetic Aβ oligomers (*14–16*) also were ineffective at inducing parenchymal and vascular amyloid, as was Aβ isolated from the conditioned media of cells stably transfected with APP (table S1; fig. S6). Addition of cofactors such as ApoE4 and Cu/Zn, which are thought to promote the polymerization of Aβ (*17, 18*), did not augment the potency of synthetic material (table S1). Injection of synthetic Aβ mixed with brain extract from wild-type mice also did not produce notable β-amyloidosis (table S1). Finally, the possibility that poor seeding by synthetic Aβ results from the activation of the Aβ-degrading enzyme neprilysin (*19*) or a humoral immune response (*20*) was ruled out by unchanged neprilysin immunoreactivity and by the absence of serum anti-Aβ antibodies in mice injected with synthetic Aβ.

Thus, cerebral extracts induce Aβ deposition in vivo by supplementing (or anticipating) endogenously generated Aβ seeds with exogenous seeds that probably consist of a form of multimeric Aβ. The host-specific morphology and distribution of the induced lesions underscore the essential role of the host in regulating pathogenesis, but the inducing agent also contributes to the pathologic phenotype. The inhibition of seeding by specific immunoneutralization of Aβ (*20*) or formic acid denaturation of the extracts (*21*) suggests that the active agent consists of an aggregated Aβ species (*20, 21*). The finding that synthetic Aβ lacks amyloid-inducing activity in vivo was not unexpected, inasmuch as prion disease has also been difficult to transmit by in vitro–generated (recombinant) prions (*8*).

Synthetic and cell culture–derived Aβ, in concentrations similar to those tested in the present study, are neurotoxic in vivo (*14, 22, 23*) and can impair long-term potentiation and cognitive function (*24, 25*). These observations, in light of the highly variable seeding efficacy of in vitro and in vivo preparations, suggest the occurrence of various Aβ conformations with partially distinct biological activities (*26*), similar to prions (*27, 28*). Polymorphic and self-propagating synthetic Aβ strains recently have been reported (*9*). Thus, Aβ multimers in vivo also may be polymorphic and polyfunctional, again reminiscent of prions, in which infectivity is strain-dependent and fully encoded in distinct multidimensional conformations (*29*). Whether oligomeric forms of Aβ that are thought to be key cytoactive disease agents (*25, 30, 31*) can also be seeded in vivo remains to be determined.

There is currently no evidence that β-amyloidosis (and in particular AD) is transmissible in the same sense as are prion diseases, which can be transmitted to wild-type hosts via diverse routes of varying efficiency and involve systemic cellular mechanisms of prion uptake and distribution (*7, 32*). However, an understanding of the mechanisms involved in the instigation and propagation of abnormal Aβ assemblies in vivo could shed light on the origins of idiopathic Alzheimer's disease.

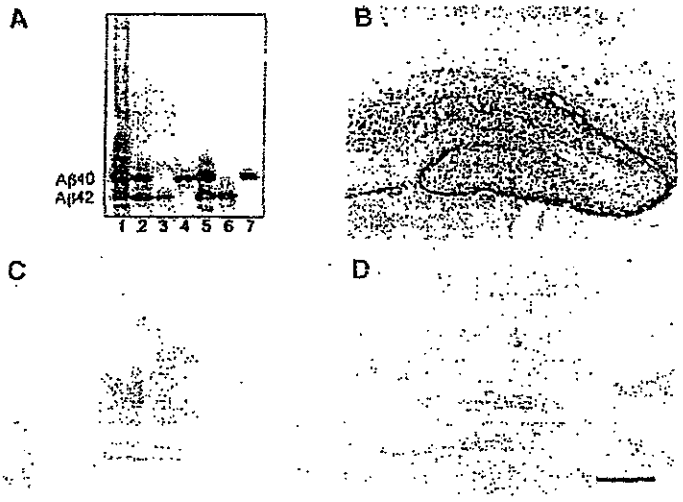


**Fig. 4.** Fresh and aged Aβ preparations were made from Aβ1-40 or Aβ1-42, or from a 2:1 mixture of Aβ1-40 and Aβ1-42 at ~5 ng/μl, similar to the Aβ concentrations in a 10% APP23 brain extract. (A) Urea-based SDS-PAGE followed by immunoblotting with a human Aβ-specific antibody. Lane 1: APP23 Tg brain extract; lane 2: mixed fresh Aβ1-40 + Aβ1-42; lane 3: fresh Aβ1-42; lane 4: fresh Aβ1-40; lane 5: aged Aβ1-40 + Aβ1-42; lane 6: aged Aβ1-42; and lane 7: aged Aβ1-40. Preparations were injected intrahippocampally into 5-month-old male APP23 mice that were analyzed 4 months later. The aged Aβ preparations were fibrillar in nature as verified by electron microscopy and Congo red binding (fig. S6). Multimeric Aβ species are not easily detected with this urea-based gel system, which is designed to separate the different Aβ isoforms. The additional band in the brain extract is likely Aβ1-38. (B) Amyloid induction with an APP23 Tg extract. (C and D) No induced Aβ deposits were detectable with any of the synthetic Aβ preparations at this concentration. Shown are animals injected with freshly mixed Aβ1-40 + Aβ1-42 (C) and aged Aβ1-40 + Aβ1-42 (D). Scale bar, 350 μm.


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**Supporting Online Material**

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Material and Methods
Fig. S1 to S6
Table S1
References


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# 医薬品 研究報告 調査報告書

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| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| ○1918年のインフルエンザウイルスのヘマグルチニンの2つのアミノ酸の変化が伝播を終焉させた<br>1918年のインフルエンザパンデミックは、世界中に広まったウイルスの感染爆発による大災害であった。本稿は、この1918年インフルエンザのヘマグルチニン受容体の結合部位のごくわずかな変化により、ウイルスの伝播性が変化したことを示す。2つのアミノ酸変異によって、ウイルスの受容体への結合しやすさがヒトのα-2,6シアル酸からトリのα-2,3シアル酸へと転換すると、フェレット間で呼吸器飛沫による感染を起こさないウイルスとなったが、致死性および上気道での複製能は保持していた。さらに、α-2,6およびα-2,3双方に特異性のある1918年ウイルスは感染性が低いことから、ヒトのalpha-2,6シアル酸への結合優先性が、このパンデミックウイルスの至適感染に不可欠であることを示している。以上の知見は、ヘマグルチニン受容体の特異性が、哺乳類におけるインフルエンザ伝播に重要な役割を担うことを裏付けるものである。 | クロスエイトM250<br>クロスエイトM500<br>クロスエイトM1000<br><br>血液を原料とすることに由来する感染症伝播等<br>vCJD等の伝播のリスク |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 1918年インフルエンザウイルスのヘマグルチニン受容体結合部位のごくわずかな変化により、ウイルスの伝播性が変化したとの報告である。 | トリインフルエンザウイルスは脂質膜を持つRNAウイルスである。これまで、本製剤によるトリインフルエンザウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれている。本製剤の安全性は確保されていると考えるが、トリインフルエンザは日本でも鶏から検出されており、流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。 |

reduced but not abolished, were not as sensitive as *rtt109Δ* cells (Fig. 3, A and B). Moreover, *rtt109Δ H3-K56R* and *rtt109Δ H3-K56A* double-mutant cells displayed similar sensitivities toward these DNA-damaging agents as either single mutant alone (Fig. 3A and fig. S5). In contrast, cells expressing *rtt109* site-specific mutants where H3-K56 acetylation was not affected were resistant to these DNA-damaging agents (Fig. 3B and fig. S6). These results suggest that the ability of Rtt109 to suppress sensitivity toward DNA-damaging agents is mainly mediated by its HAT activity toward H3-K56.

In budding yeast, Rad52 forms spontaneous foci, predominantly during S and $G_2$-M phases of the cell cycle, and these foci are thought to be sites of repair of DNA lesions (*18*, *19*). Cells with mutations in proteins involved in DNA metabolism, such as Top3 exhibit elevated levels of Rad52 foci, possibly due to an increase in spontaneous chromosome breaks (*20*). The *rtt109Δ* and *H3-K56R* single- and double-mutant cells showed a substantial increase in Rad52 fused with yellow fluorescent protein (Rad52-YFP) foci (Fig. 3, C and D). Moreover, the *rtt109Δ* H3-K56R double-mutant cells did not exhibit more Rad52 foci than either *rtt109Δ* or *H3-K56R* mutant alone (Fig. 3D). Thus, the increase in Rad52-YFP foci observed in *rtt109Δ* mutant cells appears mainly to be due to loss of H3-K56 acetylation. Supporting this idea, acetylation of four other H3 lysine residues (K9, K14, K18, and K23) was not altered in the *rtt109Δ* mutant cells (fig. S7). Taken together, these data indicate that Rtt109-mediated acetylation of H3-K56 during S phase protects DNA from damage.

Here we have shown that Rtt109 is a member of a novel HAT family that acetylates H3-K56. The *rtt109Δ* mutant exhibited a synthetic lethal or slow-growth phenotype with a mutant allele of PCNA (proliferating cell nuclear antigen), *pol30-79*, which is defective in DNA replication and repair (*21*), but not with the PCNA mutant allele, *pol30-8*, which is defective in epigenetic silencing (*22*) (fig. S8A). The *rtt109Δ* mutant also exhibited a synthetic lethal/slow growth phenotype with a mutation in DNA polymerase α (fig. S8B) and was previously found to genetically interact with Orc2 and Cdc45 mutations (*23*, *24*). All of these proteins are involved in DNA replication. The genetic interactions between Rtt109 and the proteins involved in DNA replication suggest that the *rtt109Δ* mutant cells are defective in certain aspects of DNA replication. In support of this idea, the *rtt109Δ* mutant exhibits synthetic lethal or slow-growth phenotypes with mutations in genes such as *RAD52*, which are involved in homologous recombination (*25*), a process that is needed to resolve stalled replication forks (*26*). Thus, H3-K56 acetylation by Rtt109 is closely linked to DNA replication.

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### Supporting Online Material

www.sciencemag.org/cgi/content/full/315/5812/653/DC1
Materials and Methods
Figs. S1 to S8
Tables S1 and S2
References

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# A Two–Amino Acid Change in the Hemagglutinin of the 1918 Influenza Virus Abolishes Transmission

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The 1918 influenza pandemic was a catastrophic series of virus outbreaks that spread across the globe. Here, we show that only a modest change in the 1918 influenza hemagglutinin receptor binding site alters the transmissibility of this pandemic virus. Two amino acid mutations that cause a switch in receptor binding preference from the human α-2,6 to the avian α-2,3 sialic acid resulted in a virus incapable of respiratory droplet transmission between ferrets but that maintained its lethality and replication efficiency in the upper respiratory tract. Furthermore, poor transmission of a 1918 virus with dual α-2,6 and α-2,3 specificity suggests that a predominant human α-2,6 sialic acid binding preference is essential for optimal transmission of this pandemic virus. These findings confirm an essential role of hemagglutinin receptor specificity for the transmission of influenza viruses among mammals.

The "Spanish" influenza pandemic virus spread globally and resulted in the deaths of up to 50 million people worldwide (*1*, *2*). The ability of this H1N1 pandemic strain to spread rapidly and cause high rates of illness among humans makes it valuable for studying the molecular properties that confer efficient transmissibility of influenza viruses. An influenza virus bearing all eight gene segments of the 1918 pandemic virus was recently generated in cultured cells, was found to be lethal for chicken embryos and mice, and displayed a high-growth phenotype in human lung cells. Furthermore, the 1918 hemagglutinin (HA) and polymerase genes were shown to be essential for maximal virus replication and optimal virulence (*3*–*5*).

Influenza pandemics seem to occur every 10 to 40 years, but the factors that lead to the emergence of pandemic viruses are complex and poorly understood. However, the establishment of efficient and sustained human-to-human transmission of a virus to which humans have

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little or no preexisting immunity is a fundamental property of pandemic strains (*6*, *7*). Most threatening is the possibility of another pandemic, similar to that experienced in 1918, caused by a novel influenza subtype virus capable of causing severe respiratory disease and death. The avian influenza H5N1 virus, which has resulted in more than 250 human infections (*8*), has not acquired human influenza virus genes and lacks the ability to spread efficiently from human to human (*9*, *10*). Reassortment of avian H5N1 virus genes with human H3N2 influenza virus genes was shown to be insufficient for transmission of this avian virus (*11*), suggesting that additional unknown mutations are required for H5N1 to emerge as a pandemic strain.

The binding of influenza viruses to their target cells is mediated by the viral HA, which recognizes cell surface glycoconjugates containing terminal sialic acid (SA) residues. Avian influenza viruses preferentially bind SA linked to galactose by an α-2,3 linkage (α2,3 SA), which is found in high concentrations on the epithelial cells of the intestine of waterfowl and shorebirds (*12*). Conversely, human influenza viruses (H1 to H3 subtypes) more readily bind to receptors that contain terminal α-2,6-linked sialyl-galactosyl (α2,6 SA) moieties that are found on the human respiratory tract epithelium (*13*, *14*). The three influenza pandemic viruses of the last century, occurring in 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2), each possessed an HA with a human α2,6 SA binding preference and are thought to have originated from an avian virus possessing the α2,3 SA binding preference (*13*–*16*). It has been postulated that the lack of sustained human-to-human transmission of avian influenza H5N1 viruses is due to their α2,3 SA receptor binding preference (*17*–*19*). Higher proportions of α2,3 SA receptors in the human lower respiratory tract compared with the upper respiratory tract may explain the severity of H5N1 viral pneumonia in humans resulting from H5N1 viral attachment deep in the lungs (*17*, *19*).

Amino acids at positions 190 and 225 in the 1918 pandemic influenza virus HA determine its receptor binding specificity (*15*, *16*). In this study, we generated recombinant influenza viruses possessing all eight gene segments of the 1918 influenza virus to examine the role of receptor binding specificity on replication, pathogenicity, and transmissibility of this pandemic strain. We generated two variant A/South Carolina/1/18 (SC18) 1918 viruses in which the HA was altered to change the receptor binding specificity from the parental human α2,6 SA (SC18) receptor preference to an avian α2,3 SA receptor preference (AV18) or a mixed α2,6 and α2,3 SA specificity reflecting the A/New York/1/18 (NY18) virus binding specificity. The NY18 virus was a natural variant sequenced from an archived lung tissue sample prepared during autopsy of a patient who died within 6 days of hospitalization in September 1918 (*20*). The HA corresponding to NY18 virus was made by introducing a single amino acid substitution [$Asp^{225}$→$Gly^{225}$ (D225G)] in the SC18 HA. The AV18 virus, which differs by one amino acid from NY18 virus, was made by introducing an additional amino acid change [$Asp^{190}$→$Glu^{190}$ (D190E)] within the NY18 HA. Compared with the SC18 virus, the AV18 variant has two amino acid changes (D190E and D225G) in the HA, which matches the conserved avian consensus sequence in the receptor binding site and which converts it to the classic α2,3 SA receptor preference (*15*). A/Duck/Alberta/35/76 (Dk/Alb) and A/Texas/36/91 (Tx/91) viruses were included in the study as controls representative of an avian

**Table 1.** Titer of virus stocks prepared on MDCK cells with trypsin (1 μg/ml, Sigma) and incubated at 37°C with 5% $CO_2$ for 48 hours. Hemagglutination assay of viruses used 0.5% α-2,3-resialylated CRBCs, α-2,6-resialylated CRBCs, or untreated CRBCs. The results shown correspond to four hemagglutination units. Similar results were obtained when viruses were adjusted to 8, 16, or 32 hemagglutination units with untreated CRBCs.

| | Amino acid position (H3 numbering) | | | Presence or absence of hemagglutination | | |
|---|---|---|---|---|---|---|
| | 190 | 225 | Infectivity titer (pfu/ml) | α2,6 CRBCs | α2,3 CRBCs | Untreated CRBCs |
| SC18 | D | D | $4.8 \times 10^7$ | + | − | + |
| NY18 | D | G | $3.3 \times 10^7$ | + | + | + |
| AV18 | E | G | $5.0 \times 10^7$ | − | + | + |
| Dk/Alb | E | G | $2.2 \times 10^7$ | − | + | + |

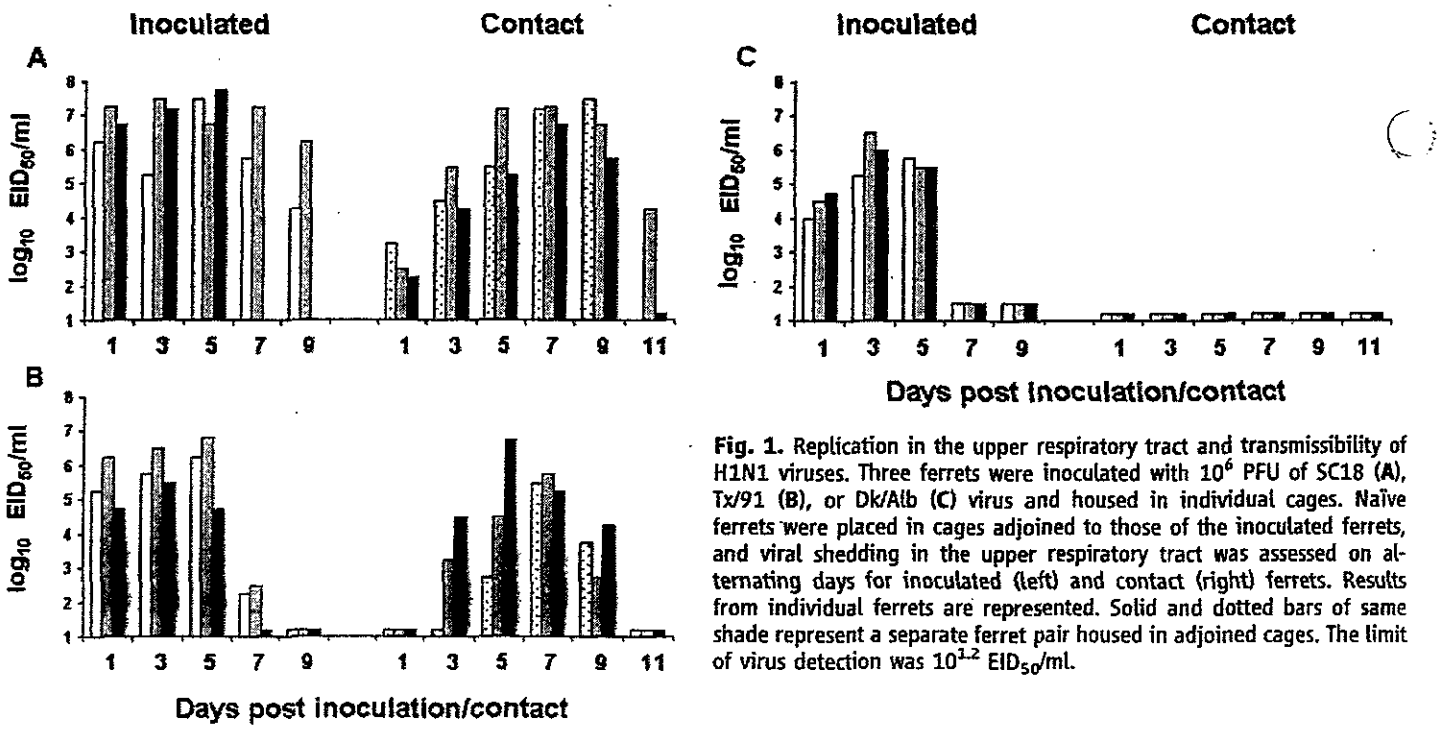


**Fig. 1.** Replication in the upper respiratory tract and transmissibility of H1N1 viruses. Three ferrets were inoculated with $10^6$ PFU of SC18 (**A**), Tx/91 (**B**), or Dk/Alb (**C**) virus and housed in individual cages. Naïve ferrets were placed in cages adjoined to those of the inoculated ferrets, and viral shedding in the upper respiratory tract was assessed on alternating days for inoculated (left) and contact (right) ferrets. Results from individual ferrets are represented. Solid and dotted bars of same shade represent a separate ferret pair housed in adjoined cages. The limit of virus detection was $10^{1.2}$ $EID_{50}$/ml.

H1N1 virus and a human H1N1 virus, respectively. The 1918 viruses were generated by using the previously described reverse genetics system (*21–23*), and the identities of virus genes in the rescued viruses were confirmed by reverse transcription polymerase chain reaction and sequence analysis.

The rescued 1918 viruses containing the parental SC18 HA and the two variant HAs had similarly high infectivity titers in Madin-Darby canine kidney (MDCK) cells (Table 1). The receptor-binding properties of the 1918 viruses were confirmed in HA assays by using enzymatically modified chicken red blood cells (CRBCs) that contain either α2,3 or α2,6 SA, as previously described (*15*). The AV18 virus and the avian Dk/Alb control virus hemagglutinated the α2,3-resialylated CRBCs only, whereas the SC18 virus hemagglutinated the α2,6-resialylated CRBCs only. The NY18 virus hemagglutinated both α2,3- and α2,6-resialylated CRBCs.

Pathogenesis and transmissibility of the parental 1918 (SC18) virus were evaluated and compared with those of Tx/91 virus with an α2,6 SA receptor binding preference (*16*) and with those of the avian Dk/Alb virus possessing an α2,3 SA receptor binding preference (Table 1) (*24*). Ferrets were housed in adjacent cages that prevented direct and indirect contact between animals but allowed spread of influenza virus through the air (*11*, *25*). They were inoculated intranasally with $10^6$ PFU (plaque forming units). One day after infection, three naïve ferrets housed in transmission cages were placed adjacent to each of the three inoculated ferrets (*26*). Three additional inoculated ferrets from each virus-infected group were killed on day 3 postinoculation (p.i.) for assessment of pathologic and virologic parameters (*26*). Ferrets inoculated with the parental SC18 virus shed high titers of infectious virus in nasal washes beginning as early as day 1 p.i. [50% egg infectious dose ($EID_{50}$/ml) from $10^{6.25}$ to $10^{7.25}$], and they sustained titers of $\geq 10^{4.5}$ $EID_{50}$/ml for 9 days p.i. (Fig. 1A, left). SC18 virus caused severe disease in all inoculated ferrets starting 2 days p.i.; symptoms included lethargy, anorexia, rhinorrhea, sneezing, severe weight loss (Table 2 and fig S1), and high fever, and two of the three animals died by day 11 p.i. Ferrets inoculated with H1N1 Tx/91 and Dk/Alb also shed high titers of virus in nasal washes (peak titers had $EID_{50}$/ml values from $10^{5.5}$ to $10^{6.8}$), but they were able to clear the virus from the upper respiratory tract by day 9 p.i. (Fig. 1, B and C) after displaying minimal symptoms (Table 2).

The human SC18 and Tx/91 viruses efficiently transmitted to each of the three contact ferrets (Fig. 1, A and B, right). The SC18 virus was detected in the contact ferrets as early as day 1 postcontact (p.c.), whereas the Tx/91 virus required 3 to 5 days to achieve detectable virus titers in nasal washes of the Tx/91 contact ferrets. The Tx/91 contact ferrets exhibited little morbidity, whereas all three SC18 contact ferrets exhibited severe signs of illness and weight loss, and one of three contact animals failed to clear the virus before it succumbed to infection on day 6 p.c. In contrast to the efficient spread of SC18 and Tx/91 viruses, the avian Dk/Alb virus was not transmitted to naïve contact ferrets, because virus was not detected in the nasal washes from the contact ferrets at any time. Furthermore, seroconversion was not detected by hemagglutination inhibition (HI) analysis of postexposure sera (Table 2). Both A/Duck/New York/15024/96 and A/Turkey/South Dakota/7034/86, which are representative avian viruses with an α2,3 SA receptor preference, exhibited efficient replication in the upper respiratory tract, but no transmission was detected between ferrets.

We introduced one– and two–amino acid substitutions into the 1918 virus HA to produce SC18 variants NY18 and AV18, respectively. A switch in receptor specificity from an α2,6 SA

**Table 2.** Clinical symptoms, virus replication, seroconversion, and transmissibility among ferrets inoculated with H1N1 viruses and among ferrets exposed to the inoculated animals (contacts). The percentage of mean maximum weight loss is shown. NW, nasal wash.

| | Inoculated ferrets<br>Number with characteristic/total number | | | | Contact ferrets<br>Number with characteristic/total number | | | Respiratory droplet transmission |
|---|---|---|---|---|---|---|---|---|
| | Sneezing (day of onset) | Weight loss (%) | Virus detected in NW | Seroconversion (range of HI antibody titer) | Weight loss (%) | Virus detected in NW | Seroconversion (range of HI antibody titer) | |
| SC18 | 3/3 (2) | 3/3 (11.7) | 3/3 | 1/1 (1280)* | 2/3 (15.4) | 3/3 | 3/3 (80–640) | Efficient |
| Tx/91 | 3/3 (2) | 3/3 (6.2) | 3/3 | 3/3 (160–640) | 3/3 (3.5) | 3/3 | 3/3 (160–320) | Efficient |
| Dk/Alb | 2/3 (5) | 2/3 (1.2) | 3/3 | 3/3 (80–1280) | 0/3 | 0/3 | 0/3 | None |
| AV18 | 0/3 | 3/3 (14.7) | 3/3 | 1/1 (640)* | 0/3 | 0/3 | 0/3 | None |
| NY18 | 0/3 | 3/3 (18.9) | 3/3 | 2/2 (320–640)† | 1/3 (1.4) | 1/3 | 2/3 (40–80) | Inefficient |

*Only one ferret survived and was tested. †Two ferrets survived and were tested.

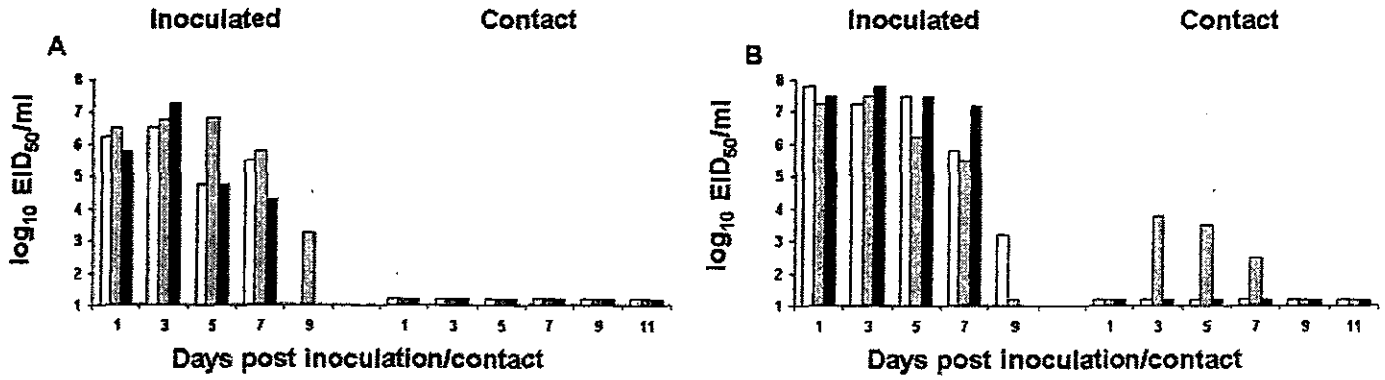


**Fig. 2.** Respiratory droplet transmissibility of 1918 viruses with mutated HA proteins. Three ferrets were inoculated with $10^6$ PFU of AV18 (**A**) or NY18 (**B**) virus and placed in separate cages. Naïve ferrets were placed in cages adjoined to those of the inoculated ferrets, and viral shedding in the upper respiratory tract was assessed on alternating days for inoculated (left) and contact (right) ferrets. Results from individual ferrets are represented. Solid and dotted bars of same shade represent a separate ferret pair housed in adjoined cages.

(human) to an α2,3 SA (avian) binding preference abolished the transmissibility of the pandemic virus (Fig. 2 and Table 2). Although ferrets inoculated with AV18 virus exhibited severe illness (Table 2 and fig S1) and shed high titers of infectious virus in nasal washes (Fig. 2A, left), none of the three AV18 contact ferrets had detectable virus in nasal washes, and post-exposure sera collected from contact animals lacked antibodies against AV18. The NY18 virus, with dual α2,6 and α2,3 SA specificity, also resulted in severe illness and death among the inoculated ferrets, but it failed to transmit efficiently, as evidenced by the paucity of clinical symptoms and virus shedding among the contact ferrets (Fig. 2B). Two of the three NY18 contact ferrets seroconverted with relatively low HI titers of 40 and 80 (Table 2). The lack of efficient transmission was not due to the inability of the NY18 virus to replicate to high titers in the upper respiratory tract, including the nasal turbinates (Fig. 2B, left, and fig S2). Interestingly, no sneezing was noted among the AV18- and NY18-inoculated ferrets through a 14-day observation period, a finding consistent with the lack of notable sneezing observed in ferrets infected with H5N1 viruses (*11*).

Despite the differences in transmissibility of the parental 1918 (SC18) virus and the mutant 1918 viruses, similar damage to multiple lung lobes was observed 3 days after intranasal infection (*26*) (Fig. 3). Ferret lungs infected with SC18, AV18, and NY18 viruses exhibited necrotizing bronchiolitis and moderate to severe alveolitis with edema (Fig. 3, A to E, I, and J). Viral antigen was common in lung tissues, with localization in the upper to lower portions of the bronchial airways, bronchial and bronchiolar epithelium, and hyperplasic epithelium within alveoli (Fig. 3, F to H). Ferrets inoculated with control Tx/91 and Dk/Alb viruses generally showed a lack of significant lung lesions (Fig. 3, K to M).

Receptor binding, the initial event in influenza virus infection, was a major determinant of virus transmission efficiency of the H1N1 pandemic virus. This work also evaluates the virulence of the 1918 virus in a ferret model, a model that is believed to be more representative than the mouse model of disease caused by influenza viruses in humans. In contrast to other human influenza virus strains, the 1918 virus demonstrated uniquely high virulence and lethality in ferrets. The mutant 1918 virus possessing α2,3 SA receptor binding (AV18) was equally virulent in ferrets as the parental SC18 strain at the dose administered. Remarkably, the AV18 virus replicated in the upper respiratory tract as efficiently as the parental SC18 virus, but it failed to transmit to contact ferrets. Moreover, a human α2,6 SA binding preference is essential for optimal transmission of this exceptionally virulent virus. The introduction of a single mutation that converts the HA to dual α2,6 and α2,3 SA binding specificity (NY18) reduced the high transmissibility observed with the parental 1918 (SC18) virus. This result is consistent with the previously demonstrated lack of transmissibility of an H5N1 2003 virus that possessed dual α2,6 and α2,3 SA specificity due to a naturally acquired mutation at HA residue 223 (H5 numbering; residue 227 by H3 numbering) (*11*, *27*).

Our findings raise the possibility that, to become more transmissible, the currently circulating avian influenza H5N1 virus may require a receptor binding change to a predominant α2,6 SA binding preference. Such a modification of H5 HA may result in improved virus binding to

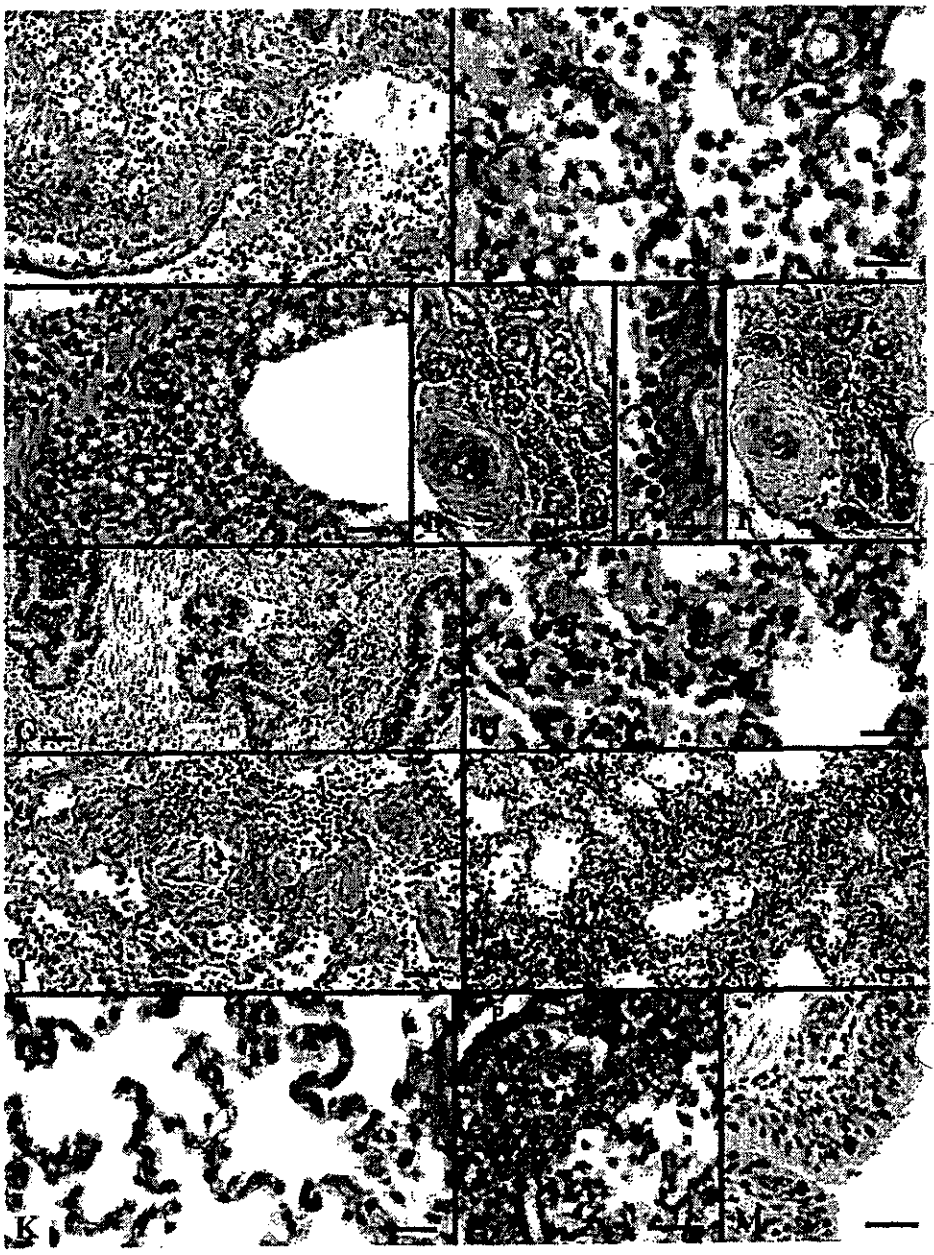

**Fig. 3.** Photomicrographs of hematoxylin and eosin [(A) to (E) and (I) to (L)] and immunohistochemically [(F) to (H) and (M)] stained lung sections from influenza virus–infected ferrets sampled on day 3 after inoculation. (**A to H**) Lung sections infected by SC18 virus. (A) Severe necrotizing bronchiolitis with severe diffuse alveolitis and edema. Scale bar indicates 50 μm. (B) Severe diffuse alveolitis; scale bar, 20 μm. (C) Necrotizing bronchiolitis; scale bar, 30 μm. (D) Necrosis and (F) associated influenza viral antigen in submucosal serous glandular epithelium of a bronchus; scale bar, 50 μm. (E) Margination and adhesion of neutrophils to endothelial cells of a pulmonary arteriole; scale bar, 20 μm. (G) Influenza viral antigen in epithelium of a primary bronchiole; scale bar, 50 μm. (H) Viral antigen commonly in macrophages and alveolar epithelial cells; scale bar, 20 μm. (I) NY18 virus; severe diffuse alveolitis with accompanying necrotizing bronchiolitis; scale bar, 50 μm. (J) AV18 virus; diffuse severe alveolitis and edema with necrotizing bronchiolitis; scale bar, 50 μm. (K) Tx/91 virus; normal alveoli; scale bar, 15 μm. (L) Dk/Alb virus, purulent bronchiolitis (p) with peribronchiolar mixed cell inflammation and associated moderate alveolitis (a); scale bar, 50 μm. (M) Dk/Alb viral antigen in bronchial epithelium; scale bar, 30 μm.

human tracheal epithelial cells expressing high amounts of terminal α2,6 SA motifs and, simultaneously, in an improved ability to overcome the inhibitory effects of human bronchial mucins associated with α2,3 SA receptors (*28*). However, mutations that caused a shift from the avian-type to human-type receptor binding specificity for the H1 subtype do not cause an equivalent shift in specificity for the H5 subtype (*24*). Likewise, the amino acid changes required to alter the H3 HA from an avian- to human-type receptor binding specificity are different from those required for the H1 HA. Therefore, it is likely that different avian HA subtypes have different structural requirements to confer receptor specificity. Thus, it is currently unknown which additional mutations in the H5 HA would cause a shift to the human-type specificity, which may be required for H5N1 viruses to transmit efficiently among humans.


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25. The use of the term "respiratory droplet transmission" throughout this report refers to transmission in the absence of direct or indirect contact and does not imply an understanding of the droplet size involved in virus spread between ferrets. The ability of each virus to undergo respiratory droplet transmission among ferrets was assessed by measuring virus titers in nasal washes from contact animals every other day for 9 days. HI analysis was also performed on postexposure ferret sera collected 18 days p.c. Although only single experiments are reported, there was little variation in the replication and transmissibility among the three inoculated and the three contact ferrets for each of the seven H1N1 viruses tested in this study.
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29. We thank C. Chesley for critical review of the manuscript and J. Beck for technical assistance. This work was partially supported by NIH grant PO1 AI058113 (to A.G.-S.), the Northeast Biodefense Center (U54 AI057158), and the Center for Investigating Viral Immunity and Antagonism (CIVIA) (U19 AI62623). Work in the A.G.-S. and P.P. laboratories is partially supported by the W. M. Keck Foundation. P.P. is a Senior Scholar of the Ellison Medical Foundation. This work was partially supported by Agriculture Research Service, USDA, Current Research Information System project number 6612-32000-039-00D.


**Supporting Online Material**

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Materials and Methods
SOM Text
Figs. S1 and S2
References


12 October 2006; accepted 13 December 2006
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# Protein Kinase C β and Prolyl Isomerase 1 Regulate Mitochondrial Effects of the Life-Span Determinant p66$^{Shc}$


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66-kilodalton isoform of the growth factor adapter Shc (p66$^{Shc}$) translates oxidative damage into cell death by acting as reactive oxygen species (ROS) producer within mitochondria. However, the signaling link between cellular stress and mitochondrial proapoptotic activity of p66$^{Shc}$ was not known. We demonstrate that protein kinase C β, activated by oxidative conditions in the cell, induces phosphorylation of p66$^{Shc}$ and triggers mitochondrial accumulation of the protein after it is recognized by the prolyl isomerase Pin1. Once imported, p66$^{Shc}$ causes alterations of mitochondrial $Ca^{2+}$ responses and three-dimensional structure, thus inducing apoptosis. These data identify a signaling route that activates an apoptotic inducer shortening the life span and could be a potential target of pharmacological approaches to inhibit aging.


The protein p66$^{Shc}$ (*1*–*4*) is an alternatively spliced isoform of a growth factor adapter that is phosphorylated upon oxidative stress (*2*). Ablation of the p66$^{Shc}$ gene causes life-span prolongation with no pathological consequence (*2*). A fraction of p66$^{Shc}$ localizes to mitochondria (*3*–*5*), where it binds to cytochrome c and acts as oxidoreductase, generating reactive oxygen species (ROS) and leading to organelle dysfunction and cell death (*5*). The route leading to p66$^{Shc}$ activation is still unclear. Phosphorylation of a critical serine (Ser$^{36}$) is necessary (*2*), but the kinase responsible has not been identified. Moreover, mitochondrial p66$^{Shc}$ is unphosphorylated, indicating that additional regulatory elements must exist.

Mitochondria receive, under stimulation by physiological agonists or toxic agents, $Ca^{2+}$-mediated inputs (*6*–*8*) that are decoded into effects as diverse as metabolic stimulation and apoptosis (*9*). $Ca^{2+}$ responsiveness is a highly sensitive readout of mitochondrial state: Partial defects in mitochondrial energization, as in mitochondrial diseases, cause defects in $Ca^{2+}$ handling by the organelle (*10*). Moreover, mitochondrial $Ca^{2+}$ uptake is modulated by regulatory proteins such as kinases. Some protein kinase C (PKC) isoforms (*11*) specifically affect the responses of mitochondrial $Ca^{2+}$ to agonists (PKCβ reduces them, whereas PKCζ enhances them) (*12*). PKCs are also proposed to be activated in conditions of oxidative stress (*13*). We therefore used aequorin to monitor cellular concentrations of $Ca^{2+}$, a green fluorescent protein with mitochondrial presequence (mtGFP) to monitor organelle structure, and other molecular tools to clarify the signaling route linking the oxidative challenge to the activation of p66$^{Shc}$ proapoptotic effect within


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別紙様式第2-1

No. 25

# 医薬品 研究報告 調査報告書

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| | | | | 2007. 2. 19 | 該当なし | |

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| 販売名(企業名) | クロスエイトM250(日本赤十字社)<br>クロスエイトM500(日本赤十字社)<br>クロスエイトM1000(日本赤十字社) | | | カナダ |

**研究報告の概要**

○問診票改訂 2006年12月18日
2006年12月18日付で、カナダ血液サービスは供血者が供血前に記入する供血記録の問診事項に一部修正を加える。
カナダ保健局の指示により、カナダ血液サービスは、ヒト以外の霊長類(サル、ヒヒ、チンパンジー、マカク、あるいはその血液や唾液など)との職業的接触に関する質問「あなたは過去のあるいは現在の仕事上サルやその体液を取り扱ったことがありますか?」を追加した。
サル泡沫状ウイルス(SFV)に関する認可された標準検査法がないため、供血者がこの質問に「はい」と答えた場合は無期限に供血延期となる。研究所で霊長類を扱う人、獣医師、動物園職員などが延期対象となるだろう。
SFVはサルやヒトにおけるいかなる疾患や健康被害とも関連していないが、最近の研究では、将来疾患や健康被害を引き起こす可能性を否定できないとされている。そのため、予防的措置としてこの質問が付け加えられた。

157

**使用上の注意記載状況・その他参考事項等**

クロスエイトM250
クロスエイトM500
クロスエイトM1000

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| カナダ血液サービスは、供血時の問診にヒト以外の霊長類との職業的接触に関する質問を追加したとの報告である。 | SFVはレトロウイルスに属する脂質膜を持つRNAウイルスである。これまで、本製剤によるSFV感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考えるが、念のため今後も情報収集に努める。なお、日本赤十字社では帰国後4週間は献血不適とし、輸入感染症の防止に努めている。 |

8

# Changes to the Donor Questionnaire

**December 18, 2006**

As of December 18th, 2006 Canadian Blood Services is making some adjustments to questions in the Record of Donation that blood donors complete prior to giving blood.

At the direction of Health Canada, Canadian Blood Services is adding a question regarding occupational contact with non-human primates such as monkeys, baboons, chimpanzees or macaques or their blood or saliva: "Have you, in your past or present job, taken care of or handled monkeys or their body fluids?"

Since there is no approved standard test for Simian foamy virus (SFV), donors who respond positively to the question will be deferred indefinitely. People who could be deferred include handlers of non-human primates in lab settings, veterinarians or zoo workers.

Although SFV has not been associated with any disease or health conditions in monkeys or humans, current research does not allow us to rule out the possibility that this virus could result in future disease or health conditions. So this question is being added as a precautionary measure.

As well Canadian Blood Services is removing or altering three additional questions on the record of donation:

- The question " In the past week, have you had a fever with headache?" is being eliminated. This question was added after an FDA recommendation about West Nile Virus. A positive response meant the donor would be deferred. However evidence suggests this question is no longer considered to be useful and is also being removed by U.S. blood operators and Héma-Quebec. (Question 3)
- The phrase "sometimes used for treatment of infertility or to promote weight loss" is being removed from the question " Have you ever taken human pituitary growth hormones, human pituitary gonadotrophin hormone." This hormone is no longer used in those treatments. (Question 7 b)
- The list of specific illnesses in Question 8 is repetitive of other questions on the form. So Canadian Blood Services is removing references to "liver problems," "convulsions," "blood pressure problems," "heart surgery," and "ulcerative colitis."

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医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年2月2日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
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| 一般的名称 | フィブリノゲン加第XIII因子 | | 研究報告の公表状況 | Rift Valley Fever Outbreak --- Kenya, November 2006--January 2007 | 公表国<br>米国 | |
| 販売名（企業名） | ボルヒール | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| （問題点：ケニアにおいてリフトバレー熱がアウトブレイクし、1月25日現在、404症例（死亡118症例）が確認されている。）<br><br>2006年12月中旬に、ケニア保健省に発熱および全身性の出血に関連した原因不明の死亡がNorth Eastern州に位置するGarissa地区から複数報告され、12月20日までに合計11例の死亡が報告された。最初の患者19例から収集した血清サンプルのうちの10例からリフトバレー（RFV）ウイルスRNAまたはRFVウイルスに対するIgM抗体が発見された。ヒトへのRVFウイルス感染は、RVFウイルス感染した動物を餌とした蚊や蚊以外の節足動物に刺されたり、ウイルス血症を有す動物、特に家畜に接触したりして起こる。North Eastern州では、家畜の死亡や原因不明な動物の流産といった報告があり、このこともRVFアウトブレイクを裏付けている。<br>アウトブレイクは複数の州および地区に拡大し、1月25日現在、症例404例（死亡118例）が確認されているが、アウトブレイクは2006年12月24日にピークを迎え、2006年12月27日以降、一日の症例数は減少傾向にある。12月27日付けでガリッサ地区における家畜のと殺が禁止され、その他の地区でもRVFが検出されたことから、その禁止は拡大された。2007年1月8日には弱毒生RVFワクチン接種が始まった。 | 記載なし |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 別紙のとおり | 今後とも関連情報の収集に努め、本剤の安全性の確保を図っていきたい。 |



19

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MMWR

Weekly

February 2, 2007 / 56(04);73-76

# Rift Valley Fever Outbreak --- Kenya, November 2006--January 2007

In mid-December 2006, several unexplained fatalities associated with fever and generalized bleeding were reported to the Kenya Ministry of Health (KMOH) from Garissa District in North Eastern Province (NEP). By December 20, a total of 11 deaths had been reported. Of serum samples collected from the first 19 patients, Rift Valley fever (RVF) virus RNA or immunoglobulin M (IgM) antibodies against RVF virus were found in samples from 10 patients; all serum specimens were negative for yellow fever, Ebola, Crimean-Congo hemorrhagic fever, and dengue viruses. The outbreak was confirmed by isolation of RVF virus from six of the specimens. Humans can be infected with RVF virus from bites of mosquitoes or other arthropod vectors that have fed on animals infected with RVF virus, or through contact with viremic animals, particularly livestock. Reports of livestock deaths and unexplained animal abortions in NEP provided further evidence of an RVF outbreak. On December 20, an investigation was launched by KMOH, the Kenya Field Epidemiology and Laboratory Training Program (FELTP), the Kenya Medical Research Institute (KEMRI), the Walter Reed Project of the U.S. Army Medical Research Unit, CDC-Kenya's Global Disease Detection Center, and other partners, including the World Health Organization (WHO) and Médecins Sans Frontières (MSF). This report describes the findings from that initial investigation and the control measures taken in response to the RVF outbreak, which spread to multiple additional provinces and districts, resulting in 404 cases with 118 deaths as of January 25, 2007.

Teams of investigators conducted patient interviews and reviewed medical records from December 1 forward in major health-care facilities in the districts from which cases were first reported. The teams detected additional cases by meeting with elders, other leaders, and health-care providers in villages where cases had been reported and in adjacent villages. Blood samples from patients with suspected RVF were collected and maintained at 39.2°F (4.0°C). Samples from NEP and surrounding areas were transported to a field laboratory established at Garissa Provincial Hospital by CDC, KEMRI, and KMOH; samples from other areas were sent to KEMRI laboratories in Nairobi and to a laboratory in Malindi that was supported by a team from Health Canada.

A suspected case was defined as acute onset of fever (>99.5°F [>37.5°C]) with headache or muscle and joint pain since December 1 in a person who had no other known cause of acute febrile illness (e.g., malaria). A probable case was defined as acute onset of fever in a person with unexplained bleeding (i.e., in stool, vomit, or sputum or from gums, nose, vagina, skin, or eyes), vision deterioration, or altered consciousness. A confirmed case was defined as a suspected or probable case with laboratory confirmation of the presence in serum of anti-RVF virus IgM by enzyme-linked immunosorbent assay (ELISA) or RVF virus RNA by reverse transcription--polymerase chain reaction (RT-PCR).

The index case was reported in Garissa District in a patient who had symptom onset on November 30, 2006. Retrospective analysis of sera collected during July—November 2006 at Garissa Provincial Hospital revealed no evidence of earlier acute RVF infections. As of January 25, 2007, a total of 404 cases of RVF had been reported in Kenya with 118 deaths, a case-fatality rate of 29%. Of the reported cases, 115 (29%) were laboratory confirmed by anti-RVF virus IgM by ELISA (64 cases, 56%) or RT-PCR (79, 69%), including 28 cases (24%) confirmed by both. Of the remaining 289 cases, 109 were classified as probable.

Of the 230 patients with available demographic information, 140 (61%) were male (Figure 1). Patients ranged in age from 4 to 85 years, with a median age of 27 years (30 years for females and 25 years for males). RVF cases were reported from three districts in NEP (Garissa [175 cases], Ijara [125], and Wajir [26]); five districts in Coast Province (Kilifi [38], Tana River [16], Malindi [eight], Isiolo [eight], and Taita Taveta [one]); two districts in Central Province (Kirinyanga [two] and Maragua [one]); one district in Rift Valley Province (Kajiado [three]); and one from Nairobi Area (Figure 2). The patient from Nairobi had traveled to NEP during the week before illness onset but was hospitalized in Nairobi. Ijara (population 79,932) and Garissa (population 420,918) districts had the highest RVF incidence rates: 156 and 42 per 100,000 population, respectively.

Among the first 97 reported cases from Garissa and Wajir districts with detailed epidemiologic information available, 71 (73%) met the probable case definition; 38 of the 62 patients who provided blood samples tested positive by IgM ELISA, RT-PCR, or both. The most frequently reported symptoms among the 97 patients were fever (100%), headache (90%), bleeding (76%), malaise (70%), muscle pain (62%), back pain (60%), vomiting (56%), and joint pain (51%).

Two thirds of the 66 patients who provided information on potential risk factors reported having an animal that was recently ill. The most frequently reported RVF risk factors during the 2 weeks preceding illness onset were drinking unboiled (raw) milk (72%); living within 100 meters of a swamp (70%); having an ill animal (67%); drinking milk from an ill animal (59%); working as a herdsman (50%); having a dead animal (50%); and slaughtering an animal (42%). Approximately 9% of patients reported contact with another ill human.

The outbreak peaked on December 24, 2006, and the number of daily cases has been declining since December 27, 2006 (Figure 3). A ban on livestock slaughtering in Garissa District went into effect on December 27 and was expanded as RVF was detected in additional districts. Vaccination of animals with live, attenuated RVF vaccine began on January 8, 2007. Prevention messages were developed in three languages (English, Kiswhali, and Somali), and public meetings (known as barazas) were held to spread information rapidly to the community. Messages also were disseminated via radio, a widely used communication medium in NEP. Village elders, chiefs, and religious leaders were consulted throughout Garissa District, leading to a district ban on the slaughter of livestock and closure of the livestock market. Health-care workers were trained to care for persons suspected to be infected with RVF virus.

Reported by: *P Nguku, S Sharif, A Omar, C Nzioka, P Muthoka, J Njau, A Dahiye, T Galgalo, J Mwihia, J Njoroge, H Limo, J Mutiso, R Kalani, A Sheikh, J Nyikal, Kenya Ministry of Health; D Mutonga, J Omollo, A Guracha, J Muindi, S Amwayi, D Langat, D Owiti, A Mohammed, Kenya Field Epidemiology and Laboratory Training Program, Kenya Ministry of Health/CDC. J Musaa, Kenya Ministry of Livestock and Fisheries Development. R Sang, Kenya Medical Research Institute. R Breiman, K Njenga, D Feikin, M Katz, H Burke, P Nyaga, M Ackers, S Gikundi, V Omballa, L Nderitu, N Wamola, R Wanjala, S Omulo, International Emerging Infections Program, Global Disease Detection Center, CDC Kenya. J Richardson, D Schnabel, S Martin, US Army Medical Research Unit-Kenya. D Hoel, H Hanafi, M Weiner, US Naval Medical Research Unit No. 3, Cairo, Egypt. J Onsongo, T Kojo, M Duale, A Hassan, M Dabaar, C Njuguna, Kenya*

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## Editorial Note:

RVF is an acute, febrile zoonotic disease caused by Rift Valley fever virus, which belongs to the family Bunyaviridae and genus *Phlebovirus*. The virus is primarily a vector-borne zoonotic pathogen. Humans acquire RVF through bites from infected mosquitoes or, more frequently, through exposure to the blood, body fluids, or tissues of animals that have been bitten by infected mosquitoes. Direct exposure to infected animals can occur during slaughter or through veterinary and obstetric procedures. RVF was first described in sheep in the early 20th century, and the virus was first isolated in humans in Kenya in 1930 (*1,2*). In livestock, RVF causes abortion and death. Livestock epizootics can occur after heavy rainfall and flooding that result in hatching of *Aedes* mosquitoes (thought to be the initial vector and inter-epizootic reservoir of RVF) and other vectors that feed on nearby mammals (*3*). Eastern Kenya experienced unusually heavy rainfall during October—December 2006, three times the average for that period during the preceding 8 years and 13 times the rainfall in 2005 (Kenya Meteorological Department, unpublished data, 2007).

Patients with RVF usually have initial signs and symptoms of influenza-like illness; less than 8% of patients subsequently have severe disease, including generalized hemorrhagic syndrome, encephalitis, or retinitis (*2*). The overall human mortality rate from RVF has been estimated at 0.5%—1.0% of those infected, but the rate is much higher among those with severe disease. The largest reported human outbreak occurred in Kenya during 1997—1998, in which an estimated 89,000 persons (based on a systematic serosurvey) were infected and 478 died; this outbreak also was centered in NEP (*3—5*). Previous RVF outbreaks among humans were not reported outside sub-Saharan Africa until 1977—1978, when approximately 18,000 persons became ill with RVF in Egypt, and in 2000, when approximately 800 persons in Saudi Arabia and 1,000 in Yemen had severe illness (*6—8*).

Like the 1997—1998 outbreak, the current outbreak was associated with heavy rainfall, which produced massive flooding in much of Kenya, and particularly in NEP. Climatic forecasting in conjunction with satellite imaging of flooded areas has been suggested as a method for predicting where and when RVF outbreaks might occur, potentially enabling earlier interventions (*9*).

Most of the cases before December 20 occurred in young men who herded livestock, perhaps because herdsmen are the first to identify and slaughter ill animals. Later in the outbreak, the distribution of cases broadened by age and sex. Young women also were overrepresented, perhaps because they handle uncooked animal products at home as they prepare meals for the family. Cases among children aged <5 years and the elderly have been rare, probably because they rarely interact with animals or handle raw animal products.

Most patients reported to KMOH had severe illness with bleeding, which likely accounts for the 29% case-fatality rate. Judging from previous studies, many mild, undetected RVF virus

infections likely occurred during this outbreak (5). Additional cases of severe disease also might have occurred in NEP but were not detected because of the inaccessibility of many areas of the province resulting from flooding. Many areas of NEP, including an entire division of Garissa District, were unreachable by road from early December to mid-January.

Since mid-January, RVF in livestock has been detected in districts surrounding Nairobi, signaling occurrence of the outbreak in new areas. Reports also have been received of livestock and humans with illness consistent with RVF across the border in Somalia, where disease assessment has been hampered by ongoing security concerns. Several international organizations are collaborating to control the spread of the outbreak within Kenya and to other countries. Travelers should take precautions when visiting RVF-affected areas. Generally, the risk for RVF infection among travelers to Kenya is low, unless they visit areas where an outbreak is occurring and are bitten by infected mosquitoes or come in contact with body fluids, uncooked tissue, or aerosols from infected livestock. No preventive RVF medications or licensed vaccines for humans exist. Travelers to affected areas should reduce their risk for infection by protecting themselves from mosquito bites and by avoiding direct contact with livestock. Specific recommendations for U.S. travelers are available at http://www.cdc.gov/travel/other/2006/rift_valley_fever_kenya.htm.

To control the outbreak, KMOH launched several interventions, some of which might have limited the public health impact of the outbreak. A ban on the slaughter of animals (including during Eid-ul-azha, a religious holiday) was imposed in NEP and strictly enforced. The Ministry of Livestock and Fisheries Development initiated a policy of vaccinating apparently unaffected herds of livestock in districts in which human or livestock RVF disease had been confirmed and also in adjacent districts; however, as of January 25, only a small proportion of livestock had been immunized. Other interventions included heightened disease surveillance among humans and animals, community mobilization, animal quarantines and restricted transport of livestock, and an integrated vector-control strategy, including indoor residual spraying and larviciding. RVF wards were established in which appropriate infection-control measures were encouraged.

Timely detection of this outbreak was aided by implementation of Integrated Disease Surveillance and Response* within most of the affected districts. A second factor contributing to timely detection was initiation of RVF laboratory-supported field surveillance of febrile patients at outpatient clinics in Garissa. Ongoing epidemiologic, entomologic, and veterinary studies related to this outbreak continue to 1) identify factors associated with severe forms of RVF illness and poor outcomes; 2) characterize the role of specific species of mosquitoes in transmitting, maintaining, and spreading RVF virus; 3) assess the economic impact of the outbreak; and 4) define the impact of livestock immunization with live, attenuated RVF veterinary vaccine on minimizing the spread of animal and human disease. Taking measures to decrease contact with mosquitoes through use of repellents and bednets and avoiding exposure to blood or tissues of animals that might be infected are important protective measures for preventing RVF. Livestock vaccination also can be an effective means of preventing cases of human RVF if adequate vaccination coverage and herd immunity are achieved.

Acknowledgments

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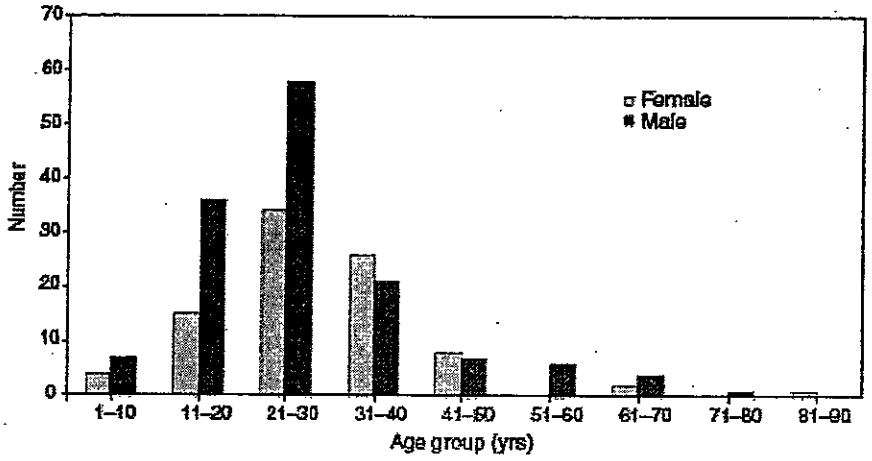
* A strategy of the African Regional Office of WHO that aims to improve availability and use of surveillance and laboratory data to control infectious diseases that are the leading causes of death, disability, and illness in the region.

Figure 1

**FIGURE 1. Number of reported Rift Valley fever cases (n = 230), by sex and age group — Kenya, November 2006–January 2007***

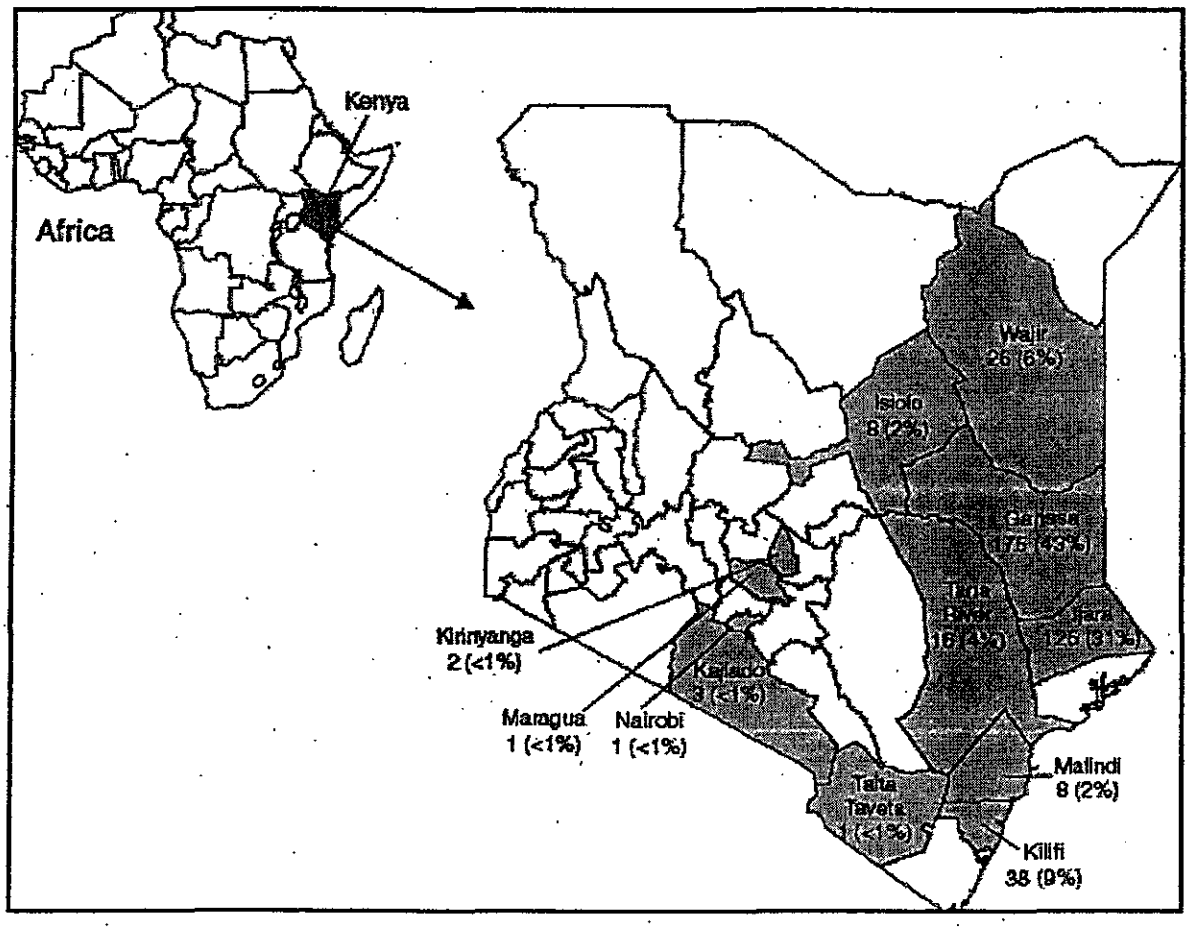



*As of January 14, 2007.

Return to top.

Figure 2

**FIGURE 2. Number and percentage of reported Rift Valley fever cases (N = 404), by district — Kenya, November 2006–January 2007***

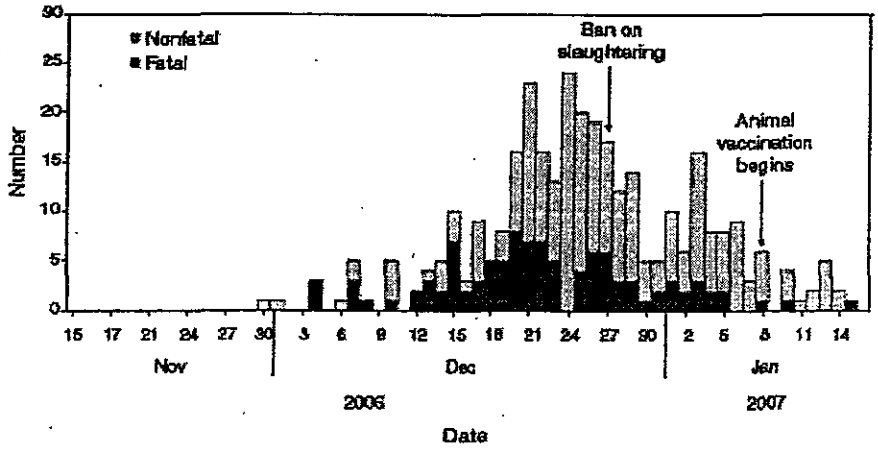



* As of January 25, 2007.

Return to top.

Figure 3

**FIGURE 3. Number of reported Rift Valley fever cases (n = 330), by date of illness onset — Kenya November 2006–January 2007***




*As of January 25, 2007, for cases with known date of onset.

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| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | ProMED 20061223-3593, 2006 Dec 23. 情報源:[1]The Yomiuri Shinbun, 2006 Dec 22. [2]Kyodo News, 2006 Dec 21. [3]Japan Times, 2006 Dec 21. [4]China Daily, 2006 Dec 19. [5]The Yomiuri Shinbun, 2006 Dec 23. | 公表国 |
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| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

○ノロウイルス最新情報－日本

[1]ノロウイルスによる感染性胃腸炎が増加している。この疾患は従来食中毒とされてきたが、昨年の症例のうち生の貝類摂食に関連したものは15%しかなく、患者の吐瀉物や排泄物から、あるいはウイルスが手を介して食物や食器に付着することで間接的に感染することが多い。予防には手洗いが重要である。寝具やカーペット、床の汚れは雑巾で丁寧に拭き取り洗浄する。洗濯は単独で手洗いし、洗濯機を汚染しないようにする。殺菌には次亜塩素酸ナトリウムと85℃以上で1分以上の加熱が有効である。不十分な場合、乾燥した布からウイルスが大気中に飛び散り、他の人がウイルスを吸入して感染するおそれがある。

[2]今シーズンのノロウイルス流行は主にヒトーヒト感染によるものであり、変異による新たなウイルス株の流行と考えられる。「急激な感染拡大は、多くの人が免疫を持たないウイルスだったためと考えられる」と専門家は話している。2006年11月27日から12月3日までの間に、全国の約3000の医療機関から65,638人の感染患者が報告された。これは1医療機関あたり21.8人で過去最高である。ノロウイルス感染は1981年から統計を実施している。例年、症例数が最高となるのは12月中旬以降である。

[3]海外旅行の際には、ウイルスや細菌による旅行者下痢症、トリインフルエンザ、デング熱やマラリア、HIV、狂犬病などに注意が必要である。旅行者は、現地の感染症や病院の場所、治療を受ける方法を確認し、事前のワクチン接種を検討すべきである。

[4]日本は胃腸炎ウイルスのアウトブレイクに見舞われている。朝日新聞は12月18日、社会福祉施設の入居者4名がノロウイルス感染により死亡したと伝えた。

[5]読売新聞の調査では、ノロウイルスによる感染性胃腸炎のため、17都道府県の小・中・高等学校少なくとも69校が学校・学級閉鎖となった。ノロウイルスは感染性が強く、短期間で感染が広まる。学校は、流行が冬休み中に収まることを期待している。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| ノロウイルスによる感染性胃腸炎が日本で流行しており、大多数は従来考えられていた食中毒ではなく、吐瀉物や排泄物を介したヒトーヒト感染であるとの報告である。 | 日本赤十字社は、問診で下痢などの体調不良者を献血不可としている。今後も引き続き情報の収集に努める。 |

**Archive Number** 20061223.3593
**Published Date** 23-DEC-2006
**Subject** PRO/EDR> Norovirus update - Japan

NOROVIRUS UPDATE - JAPAN
**********
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International Society for Infectious Diseases
<http://www.isid.org>

In this update:

[1] Decontamination instructions
[2] Node of transmission
[3] Travel advice
[4] Record high incidence
[5] School closures

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[1]
Date: Fri 22 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: The Yomiuri Shimbun, Fri 22 Dec 2006 [edited]
<http://www.yomiuri.co.jp/dy/national/20061222TDY04006.htm>

Infectious [viral] gastroenteritis caused by norovirus is spreading, and in many cases patients were infected indirectly, as they touched other patients' vomit or excretion, or the norovirus was transferred to food or tableware via people's hands. The following advice from experts at the Health, Labor and Welfare Ministry offers possible preventive measures at home and advice on how to clean up vomit from sufferers of the virus.

The disease caused by norovirus is generally known as food poisoning that [may] occur after eating such bivalve shellfish as raw oysters and clams. However, only 15 percent of cases last year were attributed to such [uncooked] shellfish. Many cases in recent years resulted from people failing to wash their hands sufficiently after touching patients' vomit or other excretions containing a large amount of the virus, or failing to thoroughly clean rooms where patients were treated. Experts assume that the surviving virus infected others through food.

The most effective preventative measure is to wash hands carefully. Antiseptic solution, ethanol and soap available in ordinary stores cannot kill noroviruses, but it is thought that washing hands well is a good way to stop the virus from becoming attached to the fingers. It is important to wash hands well with soap before meals, cooking and after going to the toilet.

If you feel nauseous before going to bed, place a container at the bed side should you have to vomit. If the bed linen is stained with vomit, remove it carefully with a cloth, taking care to collect it all. Wash the bed linen by hand, separately from other laundry. Wash carefully and avoid inhaling the spray of the washing machine water.

To [destroy] noroviruses, bleach containing sodium hypochlorite and heating clothes at 85 C or higher for at least a minute are effective. Washing clothes with water or ordinary detergents cannot remove viruses. Bleach should be diluted with water according to the

label. Use carefully, as bleach can cause some types of material to lose color or become damaged.

It is recommended to soak the laundry in boiling water, dry at high temperature, and iron it. As a futon [a type of bed mattress used in Japan] is hard to wash, iron it with a steam iron after drying it well.

Remove vomit on the floor or carpet, with a paper towel or cloth and clean the patch repeatedly with a cloth or mop. After use, the cloth should be cleaned to kill any remaining viruses. If it is insufficiently cleaned, the virus might escape into the atmosphere when the cloth dries and others inhaling the viruses may become infected. Sodium hypochlorite also is contained in kitchen bleach, toilet bleach and mold-remover for bathrooms. But cleaning the floor with cloths containing sodium hypochlorite may damage and change the color of the flooring.

During the New Year's holiday, there will be more opportunities to dine out, so if you choose bivalve shellfish from the menu, make sure they are well cooked. Soaking cooking utensils and tableware in boiling water or cleaning them with kitchen bleach also kills the virus. Shoji Miyakawa, deputy chief of the ministry's Safety Division said, "It's important for each of us to make efforts to eliminate the virus as much as possible to prevent the disease from spreading."

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[2]
Date: Thu 21 Dec 2006
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Source: Kyodo News, Thu 21 Dec 2006 [edited]
<http://search.japantimes.co.jp/cgi-bin/nn20061221a7.html>

The record-breaking nationwide outbreak of gastroenteritis caused by norovirus may stem from a mutation of the disease, an expert said Wed 20 Dec 2006. The highly contagious virus has typically been transmitted by eating raw oysters and clams, but this season's epidemic comes mostly from human-to-human infections and can best be explained by a possible outbreak of a new virus strain by, for example, mutation, said Shigeo Matsuno, a senior researcher at the National Institute of Infectious Diseases. "The cause . . . is not eating raw oysters," Matsuno said. "It must be human-to-human infection in most cases. It is natural to think that the infection spread so rapidly because many people have no immunity to the virus."

Oysters and other bivalves often cause norovirus infection because they concentrate the virus after taking it in along with plankton in the water. Apart from [uncooked] bivalve consumption, infection occurs orally when viruses in substances excreted or vomited are somehow transmitted, and in places where people are in close contiguity, such as nursing homes and schools. Oral infection with only a small number of norovirus particles -- less than 100 -- is known to cause gastroenteritis that brings stomach pain, severe diarrhea and vomiting.

Some 3000 medical institutions across Japan reported they treated 65 638 infected patients between 27 Nov and 3 Dec 2006, a record-high average of 21.8 per institution, compared with 19.8 logged in the preceding week, also a record. Norovirus infection statistics have been compiled since 1981. In previous instances, the number of cases reached a peak after mid-December.

With the rapid increase, the institute is concerned that the outbreak of norovirus gastroenteritis may show "the largest-ever spread" and is calling for people to wash their hands with soap as a way to prevent an epidemic. On Monday, Prime Minister Shinzo Abe urged Health, Welfare and Labor Minister Hakuo Yanagisawa to initiate action against the epidemic.

A public junior high school in Kitami, Hokkaido, closed Tuesday following a suspected norovirus outbreak, with 58 of the school's 416 students, and one teacher, suffering symptoms that include vomiting and abdominal pain since Saturday.

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[3]
Date: Thu 21 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: Kyodo News, via The Japan Times, Thu 21 Dec 2006 [edited]
<http://search.japantimes.co.jp/cgi-bin/nn20061221f2.html>

Travel advice
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Traveling overseas sometimes means the specter of infectious disease, such as the case of two Japanese men who died of rabies, one in November and the other in December, from separate dog bites in the Philippines. Medical experts say there are 5 major areas of concern when traveling abroad. Most conspicuous is enteritis caused by Escherichia coliform bacillus, salmonella and noroviruses. It is also called traveler's diarrhea because of its symptoms.

"About 10 percent of Japanese staying overseas for a week suffer from the disease. You should be on alert not only in developing but also in advanced countries," said Atsuo Hamada, acting director general of the Japan Overseas Health Administration Center at the Japan Labor Health and Welfare Organization.

The stomach's defenses are liable to become weaker due to fatigue from traveling, and food and drink become a source of infection. Therefore, it is important to select mineral or boiled water and to eat cooked food, he said. Influenza, colds and other respiratory infectious diseases also need caution. When it is arid, viruses easily get into the throat and the nasal mucosa. The risk rises when travelers find themselves in such arid environments as aircraft, buses and hotels, and when large numbers of people gather in a small space. Washing your hands, gargling and drinking large quantities of water are a must, said Hamada, who is also an executive of the Japanese Society of Travel and Health.

Travelers are also asked not to approach places where live birds are sold in light of concerns over avian influenza.

The 3rd source of infectious disease is the bite of a mosquito. Dengue fever, spreading chiefly in Southeast Asia and Latin America, causes headaches and joint pain, and the disease can sometimes become serious. Malaria is spreading in tropical and subtropical zones. Annually, about 100 Japanese develop symptoms of the disease after returning home, and several have died. Travelers to places where mosquitoes are a problem should not expose their hands and legs. Wear long sleeves and trousers and use an insect-repelling spray. The 4th area of concern for infections comprises sexually transmitted diseases such as HIV and syphilis and those that can be contracted at medical institutions in developing countries, including HIV and hepatitis B. "In addition to cautious deeds, travelers are asked to choose a clean hospital as far as possible," Hamada said.

The 5th concern is rabies. Because the virus is also carried by mammals other than dogs, the typical source of infection, travelers should not approach animals without caution. If bitten, they should immediately go to a hospital, he said.

"Before going abroad, travelers should confirm what infectious diseases are spreading, where hospitals are located and how to receive treatment. Depending on the circumstances, they should consider getting vaccinations beforehand," Hamada said.

[Byline: Toshiyuki Tanimoto]

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[4]
Date: Tue 19 Dec 2006
From: ProMED-mail <promedm@promedmail.org>
Source: China Daily, Xinhua News Agency report, Tue 19 Dec 2006 [edited]
<http://english.people.com.cn/200612/19/eng20061219_323888.html>

Japan has been hit by an outbreak of a common stomach virus, prompting the prime minister to call for steps to deal with

infections that have reached a record high this winter. Over 65 600 people throughout the country are thought to have been infected by norovirus between 27 Nov and 3 Dec 2006, the highest since data was first collected in 1989, according to the National Institute of Infectious Diseases. Four people living at nursing homes, 3 in their 90s, died due to the [norovirus] infection which causes severe diarrhoea and vomiting, the Asahi newspaper reported yesterday, saying all but 2 of Japan's 47 prefectures have issued warnings of a potential epidemic.

"Prime Minister (Shinzo) Abe has given instructions to the relevant ministries to prevent the spread of the infection," Chief Cabinet Secretary Yasuhisa Shiozaki told a news conference. The Health Ministry says there are no vaccines against the virus and advises people to wash their hands carefully before eating and after using the toilet. Shares in companies that make products to protect against norovirus have shot up on the Tokyo stock exchange, with hand wash and gargle maker Meiji Seika Kaisha Ltd jumping 11.5 per cent in the past week. Other beneficiaries include clinical reagents maker Eiken Chemical Co, whose have risen 14.8 per cent in a week.

The ministry said shellfish including oysters should be thoroughly cooked to destroy the virus, as it is often found in such seafood. Norovirus has caused mass infections in other countries, including the United States, where hundreds of passengers on a cruise ship fell ill to it in 2 separate incidents in early December and in November.

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[5]
Date: Sat 23 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: The Yomiuri Shimbun, Sat 23 Dec 2006 [edited]
<http://www.yomiuri.co.jp/dy/national/20061223TDY02003.htm>

At least 69 primary, middle and high schools in 17 prefectures have temporarily suspended school or classes since November, as gastroenteritis caused by the highly infectious norovirus continues to spread, according to a Yomiuri Shimbun survey of prefectural boards of education.

Norovirus is highly infectious and spreads rapidly. Schools hope the epidemic will taper off during winter holidays. Seventeen schools in Hokkaido and Akita, Fukushima, Ibaraki, Nagano and Niigata prefectures have closed temporarily, including 12 primary schools, 2 middle schools, 2 high schools and one school for disabled children. Six primary schools and a high school of 5 prefectures temporarily suspended all classes for one grade. Forty-five primary and middle schools in 12 prefectures also suspended classes temporarily.

However, the actual situation could be even worse than reported because prefectural boards of education are aware only of cases reported to them by municipal boards of education -- which are not obligated to report such cases to prefectural boards of education.

In Niigata Prefecture, 5 schools closed entire schools or classes for certain grades. A Nagaoka primary school recorded the prefecture's highest number of the gastroenteritis victims when 72 of its 410 students called in sick on 30 Nov. Sixty-one students who came to school also complained of stomachaches, and 30 percent of all students showed symptoms of gastroenteritis.

Norovirus was detected in some of the students. Because no outbreak was reported at middle schools that use the same school meal provision service, the epidemic likely was not food-borne, but an infection that spread among the students. The primary school was closed the next day and resumed usual classes on 5 Dec 2006. However, new cases have continued to appear even as recently as Monday.

In Odate, Akita Prefecture, food poisoning from meals provided through a school food service has struck many students and staff. Primary and middle schools in the city reported a surge in absent students from 14 Dec, and 8 schools closed temporarily on the afternoon on 15 Dec. According to the city board of education, 443 students, teachers and school staff had developed infectious

gastroenteritis caused by norovirus as of Thursday [21 Dec 2006]. The Odate Health Care Center traced the cause of the food poisoning to bread that was on the menu of all the schools, and norovirus was detected in an employee of the company that made the bread. Some infected students apparently passed on the virus to their families.

In Saitama Prefecture, primary schools in Ageo, Honjo and Kawagoe closed classes temporarily. The prefectural board of education instructed municipal boards of education to provide students with advice about hygiene. But despite these efforts, the epidemic has continued in many areas since.

The Education, Science and Technology Ministry told prefectural boards of education to take steps to ensure adequate hygiene levels are maintained at schools.

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[The extent of the current norovirus outbreak in Japan appears to be unprecedented, provoking both the intervention of the Prime Minister and causing perturbations in the Tokyo stock-market. - Mod.CP]

[see also:
Norovirus - Japan 20061217.3543
Norovirus, increased activity - Europe 20061214.3522
Norovirus, oysters - USA (OR) ex Korea 20061213.3503
Norovirus - Japan (multi-prefecture) 20061209.3479]
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# Ministry offers advice on norovirus

The Yomiuri Shimbun

Infectious gastroenteritis caused by norovirus is spreading, and in many cases patients were infected indirectly, as they touched other patients' vomit or excretion, or the norovirus was transferred to food or tableware via people's hands.

The following advice from experts at the Health, Labor and Welfare Ministry offers possible preventive measures at home and advice on how to clean up vomit from sufferers of the virus.

The disease caused by norovirus is generally known as food poisoning that occurs after eating such bivalve shellfish as raw oysters and clams.

However, only 15 percent of cases last year were attributed to such shellfish. Many cases in recent years resulted from people failing to wash their hands sufficiently after touching patients' vomit or other excretions containing a large amount of the virus, or failing to thoroughly clean rooms where patients were treated.

Experts assume that the surviving virus infected others through food.

The most effective preventative measure is to wash hands carefully.

Antiseptic solution, ethanol and soap available in ordinary stores cannot kill the norovirus, but it is thought that washing hands well is a good way to stop the virus from becoming attached to the fingers.

It is important to wash hands well with soap before meals, cooking and after going to the toilet.

If you feel nauseous before going to bed, place a container at the bed side should

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47. Learning fi
The Magic of
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Info
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you have to vomit.

If the bed linen is stained with vomit, remove it carefully with a cloth, taking care to collect it all. Wash the bed linen by hand, separately from other laundry. Wash carefully and avoid inhaling the spray of the washing machine water.

To kill noroviruses, bleach containing sodium hypochlorite and heating clothes at 85 C or higher for at least a minute are effective.

Washing clothes with water or ordinary detergents cannot remove viruses.

Bleach should be diluted with water according to the label. Use carefully, as bleach can cause some types of material to lose color or become damaged.

It is recommended to soak the laundry in boiling water, dry at high temperature, and iron it.

As a futon is hard to wash, iron it with a steam iron after drying it well.

Remove vomit on the floor or carpet, with a paper towel or cloth and clean the patch repeatedly with a cloth or mop.

After use, the cloth should be cleaned to kill any remaining viruses. If it is insufficiently cleaned, the virus might escape into the atmosphere when the cloth dries and others inhaling the viruses may contract the disease.

Sodium hypochlorite also is contained in kitchen bleach, toilet bleach and mold-remover for bathroom. But cleaning the floor with cloths containing sodium hypochlorite may damage and change the color of the flooring.

During the New Year's holiday, there will be more opportunities to dine out, so if you choose bivalve shellfish off the menu, make sure they are well cooked.

Soaking cooking utensils and tableware in boiling water or cleaning them with kitchen bleach also kills the virus.

Shoji Miyakawa, deputy chief of the ministry's Safety Division said, "It's important for each of us to make efforts to eliminate the virus as much as possible to prevent the disease from spreading."

Information about noroviruses in English is available at the Web site of the National Institute of Infectious Diseases (www.nih.go.jp/niid/index-e.html) and at the ministry Web site (www.mhlw.go.jp/english/index.html).

(Dec. 22, 2006)

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Govt aims to expand preventive care for seniors (Dec.28)

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# Mutation likely behind virus spread

Kyodo News

The record-breaking nationwide outbreak of gastroenteritis caused by norovirus may stem from a mutation of the disease, an expert said Wednesday.

The highly contagious virus has typically been transmitted by eating raw oysters and clams, but this season's epidemic comes mostly from human-to-human infections and can best be explained by a possible outbreak of a new virus strain by, for example, mutation, said Shigeo Matsuno, a senior researcher at the National Institute of Infectious Diseases.

"The cause . . . is not eating raw oysters," Matsuno said. "It must be human-to-human infection in most cases. It is natural to think that the infection spread so rapidly because many people have no immunity to the virus."

Oysters and other bivalves often cause norovirus infection because they concentrate the virus after taking it in along with plankton in the water.

Apart from bivalve consumption, infection occurs orally when viruses in substances excreted or vomited are somehow transmitted, and in places where people are in close contiguity, such as nursing homes and schools.

Oral infection with only a small number of norovirus organisms — less than 100 — is known to cause gastroenteritis that brings stomach pain, severe diarrhea and vomiting.

Some 3,000 medical institutions across Japan reported they treated 65,638 infected patients between Nov. 27 and Dec. 3, a record-high average of 21.8 per institution, compared with 19.8 logged in the preceding week, also a record. Norovirus infection statistics have been compiled since 1981.

In previous instances, the number of cases reached a

peak after mid-December.

With the rapid increase, the institute is concerned that the outbreak of norovirus gastroenteritis may show "the largest-ever spread" and is calling for people to wash their hands with soap as a way to prevent an epidemic.

On Monday, Prime Minister Shinzo Abe urged Health, Welfare and Labor Minister Hakuo Yanagisawa to initiate action against the epidemic.

A public junior high school in Kitami, Hokkaido, closed Tuesday following a suspected norovirus outbreak, with 58 of the school's 416 students, and one teacher, suffering symptoms that include vomiting and abdominal pain since Saturday.

The Japan Times: Thursday, Dec. 21, 2006
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# Travelers urged to gird for infectious ills

## Experts counsel common-sense precautions against menacing diseases

By TOSHIYUKI TANIMOTO
Kyodo News

Traveling overseas sometimes means the specter of infectious disease, such as the case of two Japanese men who died of rabies, one in November and the other in December, from separate dog bites in the Philippines.



The Anopheles, a species of mosquito, can transmit malaria into humans. PHOTO COURTESY OF U.S. CENTERS FOR DISEASE CONTROL AND PREVENTION/KYODO

Medical experts say there are five major areas of concern when traveling abroad. Most conspicuous is enteritis caused by Escherichia coliform bacillus, salmonella and norovirus. It is also called traveler's diarrhea because of its symptoms.

"About 10 percent of Japanese staying overseas for a week suffer from the disease. You should be on alert not only in developing but also in advanced countries," said Atsuo Hamada, acting director general of the Japan Overseas Health Administration Center at the Japan Labor Health and Welfare Organization.

The stomach's defenses are liable to become weaker due to fatigue from traveling, and food and drink become a source of infection. Therefore, it is important to select mineral or boiled water and to eat cooked food, he said.

Influenza, colds and other respiratory infectious diseases also need caution. When it is arid, viruses easily get into the throat and the nasal mucosa.

The risk rises when travelers find themselves in such arid environments as aircraft, buses and hotels, and when large numbers of people gather in a small space. Washing your hands, gargling and drinking large quantities of water are a must, said Hamada, who is also an executive of the Japanese Society of Travel and Health.

Travelers are also asked not to approach places where live birds are sold in light of concerns over avian influenza.

The third source of infectious disease is the bite of a mosquito. Dengue fever, spreading chiefly in Southeast Asia and Latin America, causes headaches and joint pain, and the disease can sometimes become serious.

Malaria is spreading in tropical and subtropical zones. Annually, about 100 Japanese develop symptoms of the disease after returning home, and several have died.

Travelers to places where mosquitoes are a problem should not expose their hands and legs. Wear long sleeves and trousers and use an insect-repelling spray.

The fourth area of concern for infections comprises sexually transmitted diseases such as HIV and syphilis and those that can be contracted at medical institutions in developing countries, including HIV and hepatitis B.

"In addition to cautious deeds, travelers are asked to choose a clean hospital as far as possible," Hamada said.

The fifth concern is rabies. Because the virus is also carried by mammals other than dogs, the typical source of infection, travelers should not approach animals without caution. If bitten, they should immediately go to a hospital, he said.

"Before going abroad, travelers should confirm what infectious diseases are spreading, where hospitals are located and how to receive treatment. Depending on the circumstances, they should consider getting vaccinations beforehand," Hamada said.

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# Outbreak of stomach virus hits Japan

Japan has been hit by an outbreak of a common stomach virus, prompting the prime minister to call for steps to deal with infections that have reached a record high this winter.

Over 65,600 people throughout the country are thought to have been infected by norovirus between November 27 and December 3, the highest since data was first collected in 1989, according to the National Institute of Infectious Diseases.

Four people living at nursing homes, three in their 90s, died due to the infection which causes severe diarrhoea and vomiting, the Asahi newspaper reported yesterday, saying all but two of Japan's 47 prefectures have issued warnings of a potential epidemic.

"Prime Minister (Shinzo) Abe has given instructions to the relevant ministries to prevent the spread of the infection," Chief Cabinet Secretary Yasuhisa Shiozaki told a news conference.

The Health Ministry says there are no vaccines against the virus and advises people to wash their hands carefully before eating and after using the toilet.

Shares in companies that make products to protect against norovirus have shot up on the Tokyo stock exchange, with hand wash and gargle maker Meiji Seika Kaisha Ltd jumping 11.5 per cent in the past week.

Other beneficiaries include clinical reagents maker Eiken Chemical Co, whose have risen 14.8 per cent in a week.

The ministry said shellfish including oysters should be thoroughly cooked to destroy the virus, as it is often found in such seafood.

Norovirus has caused mass infections in other countries, including the United States, where hundreds of passengers on a cruise ship fell ill to it in two separate incidents in early December and in November.

*Source: China Daily*

**People's Daily Online — http://english.people.com.cn/**

# Virus forcing schools to halt classes

The Yomiuri Shimbun

At least 69 primary, middle and high schools in 17 prefectures have temporarily suspended school or classes since November, as gastroenteritis caused by the highly infectious norovirus continues to spread, according to a Yomiuri Shimbun survey of prefectural boards of education.

Norovirus is highly infectious and spreads rapidly. Schools hope the epidemic will taper off during winter holidays.

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Six primary schools and a high school of five prefectures temporarily suspended all classes for one grade. Forty-five primary and middle schools in 12 prefectures also suspended classes temporarily.

However, the actual situation could be even worse than reported because prefectural boards of education are aware only of cases reported to them by municipal boards of education--which are not obligated to report such cases to prefectural boards of education.

In Niigata Prefecture, five schools closed entire schools or classes for certain grades. A Nagaoka primary school recorded the prefecture's highest number of the gastroenteritis victims when 72 of its 410 students called in sick on Nov. 30. Sixty-one students who came to school also complained of stomachaches, and 30 percent of all students showed symptoms of gastroenteritis.

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THE YOMIU
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epidemic likely was not food poisoning, but an infection that spread among the students.

The primary school was closed the next day and resumed usual classes on Dec. 5. However, new cases have continued to appear even as recently as Monday.

"There were no sick students until Nov. 29," a city board of education official said. "I was surprised at the infectiousness of norovirus."

In Odate, Akita Prefecture, food poisoning from meals provided through a school food service has struck many students and staff.

Primary and middle schools in the city reported a surge in absent students from Dec. 14, and eight schools closed temporarily on the afternoon on Dec. 15. According to the city board of education, 443 students, teachers and school staff had developed infectious gastroenteritis caused by norovirus as of Thursday.

The Odate Health Care Center traced the cause of the food poisoning to bread that was on the menu of all the schools, and norovirus was detected in an employee of the company that made the bread.

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(Dec. 23, 2006)

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# 医薬品 研究報告 調査報告書

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| | | | | 2007. 1. 10 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | ProMED 20070106-0058, 2007 Jan 6. 情報源:[1]Dr. S. K. Sharif, 2007 Jan 5. [2]Reuters Foundation AlertNet, 2007 Jan 6. | ケニヤ |

**研究報告の概要**

○リフトバレー熱、ケニア
[1]最新情報:疫学データ
2006年12月23日、ガリッサの公立病院に入院した患者複数の症例から、リフトバレー熱のヒト感染アウトブレイクが確認された。IgM及びPCRによりリフトバレー熱ウイルス感染が確定診断された。
ガリッサ地区での発病率は、19/10万、最高値は最初に患者が見つかったShanta Abakの129/10万である。症例数は2007年1月5日現在で188例に達し、うち68例が死亡した。リフトバレー熱の一般的な症状は、高熱、頭痛、筋肉痛、関節痛である。重症化した患者では、吐血や鼻出血、歯肉からの出血などの出血傾向が見られる。政府はケニヤ北東部で動物の検疫を開始し、家庭での動物の屠殺を禁止した。保健省は国際機関と協力して、病気の動物に触れない、生のミルクを飲まない、病気の、あるいは死んだ動物の肉を食べないなどの健康教育プログラムを開始した。
[2]2007年1月4日、ケニヤ北東部のIjara地区でリフトバレー熱の新規疑い例8例が国境なき医師団によって発見された。1人は死亡し、2006年12月7日のアウトブレイク開始以来67人目の死亡例となった。リフトバレー熱は稀なウイルス性疾患で、感染した動物の血液や体液、組織との接触や蚊の媒介によってヒトに感染する。感染した蚊の卵が河の土手周辺で休眠しており、洪水などで水につかると蚊となってウイルス感染を拡大するようになる。この地域で発生した洪水が原因で感染が拡大した。また、洪水のために感染地域や病院への道路が寸断されていることも問題である。蚊が増加してマラリアなど他の感染症が広がることも懸念されている。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ケニヤ北東部の洪水被害を受けた地域でリフトバレー熱の感染が拡大しているとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

**Archive Number** 20070106.0058
**Published Date** 06-JAN-2007
**Subject** PRO/AH/EDR> Rift Valley fever - Kenya (multi-province)

RIFT VALLEY FEVER - KENYA (MULTI-PROVINCE)
**********

A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1]
Date: Fri 5 Jan 2007
From: Dr. S. K. Sharif <sksharif@africaonline.co.ke>

Update: Preliminary epidemiological data
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The outbreak of Rift Valley Fever in humans was first confirmed from cases presenting with fever, headache, myalgia and hemetemis in patients admitted to Provincial General Hospital, Garissa on 23 Dec 2006. The diagnosis was confirmed as Rift Valley fever virus (RVFV) infection by specific IgM [assay] and PCR [amplification]. After the first cases were confirmed the Ministry of Health (MOH), Centre for Disease Control (CDC) and the World Health Organization sent a team to investigate and put in place control measures to stop the spread of the disease.

Preliminary data show that 71 percent of all cases admitted with RVF were males and 29 percent were females. Patients less then 20 years comprised less then 3 percent. The age of male patients was 28 years while the mean age of female patients was 33 years. The attack rate for Garissa district was 19 per 100 000 people and the highest attack rate was in Shanta Abak at 129 cases per 100 000. The first cases were reported from Shanta Abak.

The commonest presenting features were fever, headache, muscle pains and joint pains. The patients with severe disease had a bleeding tendency such as hemetemesis, bleeding from the nose and gums. A total of 86 percent of the patients interviewed said that they drank raw milk and 71 percent said that they had drunk milk from a sick animal 2 weeks before onset of symptoms. Most patients lived near a swampy area. In all, 71 percent of patients had sick animals in their herds and 60 percent had lost an animal due to disease 2 weeks prior to onset of symptoms; 53 percent had slaughtered a sick animal while 53 percent reported they had eaten meat from either a sick or a dead animal. Fifty-one percent of patients were using a [mosquito] net.

Cases have now been reported from other parts of North Eastern Province such as Ijara, Garissa and also in 2 districts of Coast Province (Tana River and Kilifi District). Cumulative figures as of 5 Jan 2007 are 188 cases with 68 deaths.

The Government has imposed a quarantine for animals in North Eastern Province and also imposed a ban on slaughtering animals at home. The Ministry of Health with CDC, WHO, UNICEF and the Red Cross have mounted an intensive health education program using electronic media, religious leaders and health workers to tell people to avoid contact with sick animals, to stop drinking raw milk and to stop eating meat from sick or dead animals. It has started distributing insecticide treated nets and indoor residual spraying and larviciding stagnant pools of water.

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Dr. S. K. Sharif
<sksharif@africaonline.co.ke>

******
[2]
Date: Sat 6 Jan 2007
From: Mary Marshall <tropical.forestry@btinternet.com>
Source: Reuters Foundation AlertNet, ex MSF Belgium [edited]
<http://www.alertnet.org/thenews/fromthefield/msfbelgium/116801382768.htm>

On [Thu] 4 Jan 2007, 8 new suspected cases of Rift Valley fever were discovered by Medecins Sans Frontieres (MSF) teams in the Ijara District in the North-Eastern Province of Kenya. One of the patients died, taking the death toll to at least 67 since the outbreak began on 7 Dec 2006. MSF started battling the outbreak on 22 Dec 2006 when teams began work in the town of Garissa, setting up facilities to care for patients in the hospital in which 23 people had been treated for their Rift Valley fever infection. Today, MSF teams are working in Garissa, Ijara and Tano River providing information, trying to locate infected people and treating patients.

Rift Valley fever is a rare viral disease of which very little is known. Transmitted primarily to humans through contact with infected animal matter, such as blood or other fluids, or organs, it is also spread from livestock via _Aedes_ mosquitoes. Consumption of raw milk, an important element in the diet of many nomadic pastoralists of the area, is also thought to lead to infection.

The epidemic has been triggered by extensive flooding in the region. [A map of the flooded areas of Kenya, where conditions may be favorable for the spread of Rift Valley fever, is available at:
<http://www.reliefweb.int/rw/fullMaps_Af.nsf/luFullMap/C1A04D85AE2299308525722
- Mod.CP]

The infected mosquito eggs are often laid around river banks and can lay dormant for years until they get submerged. Once covered with water, the eggs become infected mosquitoes which spread the virus. The last large outbreak in the same region was in 1997, also following heavy rains. During that time in Garissa District, around 27 500 were infected and 170 died. Only about one percent of people contracting Rift Valley fever develop the disease in a severe form. But of those who do, around half will die.

"The great majority of people infected just suffer from headaches and influenza-like symptoms reminiscent of malaria," explains MSF emergency coordinator Dr Ian Vanenglegem, "but the severe form, like other haemorrhagic diseases, attacks the liver and can cause the patient to bleed from every orifice. There is no cure, so we are only able to treat the symptoms."

One of the biggest challenges of dealing with this outbreak is logistical. "Much of Kenya's North-Eastern Province is not accessible by road because of the floods, so the only way we can find patients is to travel by helicopter," continues Dr Vanenglegem. [An interactive map is available at
<http://www.alertnet.org/map/index.htm?ct=2&style=2&ex_iso=ET,KE,SO,SD,TZ,UG&p
- Mod.CP]

"Just to reach the Masalani hospital in Ijara can take up to 3 days by road. It is estimated that up to 500 000 people are at risk from infection and this population is scattered over a vast area. We are sure that the number of cases discovered is only the tip of the iceberg."

Another difficulty is the fear of the outbreak among the population. With such a high death rate among those contracting the severe form of the disease, many people will see no benefit in making the often long journey to a health centre. To counter this, MSF teams are undertaking awareness-raising activities to increase understanding of what Rift Valley fever is and what measures people should take themselves. "This is an important concern because it is likely that, with the flood waters being a perfect breeding ground for mosquitoes, this outbreak will be followed to by high numbers of malaria infections. The initial symptoms may be similar to those of Rift Valley fever. If people are too scared to come to health centres, or simply don't see the point, then there may be even more unnecessary deaths," concludes Dr Vanenglegem.

--

Mary Marshall
<tropical.forestry@btinternet.com>

[ProMED-mail acknowledges contribution of the same report by Pablo Nart. - Mod.CP]

[The outbreak of Rift Valley fever in the North Eastern Province of Kenya continues to develop. The number of confirmed deaths has risen from the 54 reported on Wed 3 Jan 2007, to 67 reported by MSF on Thu 4 Jan 2007, and now to the 68 reported by Dr. Sharif in the above report. Dr. Sharif also reports that the outbreak is now affecting 2 districts of the Coast Province of Kenya as well as the initial 3 districts of North Eastern province (see map at <http://en.wikipedia.org/wiki/Image:Kenya-relief-map-towns.jpg>. Preliminary data suggest that unusually the disease is spreading in the human population through the consumption of raw milk from sick animals and that transmission by mosquitoes may not yet be a determining factor. This circumstance may enable the local authorities to control the outbreak in the human population by vector control measures. - Mod.CP]

[see also:
Rift Valley fever - Kenya (North Eastern Province) (02) 20070103.0019
Rift Valley fever - Kenya (North Eastern Province) 20070101.0004
2006
---
Rift Valley fever - Kenya (Garissa) (05) 20061228.3643
Rift valley fever - Kenya (Garissa) (03): WHO 20061228.3630
Rift Valley fever - Kenya (Garissa) (02): conf. 20061223.3590
Rift Valley fever - Kenya (Garissa): conf. 20061222.3584
Viral hemorrhagic fever - Kenya (Garissa): susp 20061221.3578
2004
---
Rift Valley fever - Saudi Arabia (Jizan) (09): OIE 20041003.2723
Rift Valley fever - Saudi Arabia (Jizan): suspected 20040907.2500
2003
---
Rift Valley fever, sheep, goat - Senegal: OIE 20031122.2901
Rift Valley Fever - Mauritania: OIE 20031020.2635
Rift Valley fever, human - Egypt (02) 20030902.2205
Rift Valley fever - Egypt 20030827.2158
Rift Valley fever, livestock, human - Mauritania: OIE 20030105.0034
2002
---
Rift Valley fever, sheep, goat - Senegal: OIE (02) 20021226.6130
Rift Valley fever, sheep and goat - Senegal: OIE 20021221.6107
1998
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Obituary: Louise Martin, CDC (Nairobi) (02) 19980811.1577
Rift Valley fever - Kenya & Somalia (07) 19981228.2447
Rift Valley fever - Kenya 19980721.1373
Rift Valley fever - Kenya & Somalia (04) 19980413.0676
Rift Valley fever - Kenya (Nairobi) 19980217.0309
Rift Valley fever - Kenya and Somalia (03) 19980131.0200]
.....cp/pg/dk

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# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 2. 16 | 該当なし | |

| 一般的名称 | 乾燥濃縮人血液凝固第Ⅷ因子 | 研究報告の公表状況 | ProMED 20070216-0586, 2007 Feb 16. 情報源:EMS Network News, 2007 Feb 15. | 公表国 オーストラリア |
|---|---|---|---|---|
| 販売名(企業名) | クロスエイトM250(日本赤十字社)<br>クロスエイトM500(日本赤十字社)<br>クロスエイトM1000(日本赤十字社) | | | |

**研究報告の概要**

○クンジンウイルス―オーストラリア(西オーストラリア)
保健当局は、Kimberley地区東部とPilbara地区東部において蚊が媒介するウイルスの証拠が見つかったとして、西オーストラリア州北部に居住あるいは滞在中の人々に、蚊に注意するよう呼びかけた。西オーストラリア大学が実施するサーベイランスプログラムによって、今年の雨期に初めてクンジンウイルスが確認された。
クンジンウイルスは、蚊によって媒介されるウイルスで、マレーバレー脳炎(MVE)ウイルスと同じグループに属する。感染すると、関節痛、発熱、発疹などロスリバーウイルスに似た症状を引き起こす。しかし、稀にMVEのように頭痛、首のこわばり、発熱、精神症状、昏睡などを発症し重症となる例もある。ウイルスに感染しやすいのは、乳児、小児、旅行者、新規雇用者など、最近感染地域に来た人々であるが、上記のような症状を発症した人は、早めに医師の診察を受けるべきである。クンジン、MVE、ロスリバーウイルスなどに対して個別の治療法やワクチンはない。
KimberleyやPilbaraの訪問を計画している場合は、予定を変更する必要はないが、肌を露出しない、DEETなどの虫除け剤を使用する、蚊を通さない網戸を取り付ける、適切な衣服や蚊帳を使用して子どもを守るなどの対策を行い、蚊に刺されないようにすることが重要である。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 今年の雨期に初めてクンジンウイルスが確認され、当局は西オーストラリア州北部に居住あるいは滞在中の人々に、蚊に注意するよう呼びかけたとの報告である。 | クンジンウイルスはウエストナイルウイルスと近縁な脂質膜を持つRNAウイルスである。これまで、本製剤によるクンジンウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考えるが、念のため今後も情報収集に努める。なお、日本赤十字社では帰国後4週間は献血不適とし、輸入感染症の防止に努めている。 |

**使用上の注意記載状況・その他参考事項等**

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クロスエイトM500
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血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

(12)

Back

**Archive Number** 20070216.0586
**Published Date** 16-FEB-2007
**Subject** PRO/EDR> Kunjin virus - Australia (WA)

KUNJIN VIRUS - AUSTRALIA (WESTERN AUSTRALIA)
**********

A ProMED-mail post
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International Society for Infectious Diseases
<http://www.isid.org>

Date: 15 Feb 2007
From: Joseph Dudley <jdudley@eaicorp.com>
Source: EMS Network News
<http://www.bigmedicine.ca/australianz.htm#Mosquito-borne_disease_risk_in_th>

The Department of Health [of Western Australia] today urged people living and holidaying in the north of Western Australia to take extra care against mosquito bites,following evidence of mosquito-borne viruses in the east Kimberley and east Pilbara regions.

Department of Health Medical Entomologist Dr Mike Lindsay said that the Department□surveillance program (undertaken by The University of Western Australia) had detected activity of Kunjin virus for the first time this wet season.

"Kunjin virus, which is carried by mosquitoes, belongs to the same group of viruses as Murray Valley encephalitis (MVE) virus," he said. [And is a member of the Japanese encephalitis complex of viruses. - Mod. TY]

"Infection with Kunjin virus can cause symptoms that are similar to Ross River virus disease, such as swollen and aching joints, fever and rash. However, in rare cases it can cause more severe symptoms, like MVE, which include headache, neck stiffness, fever, delirium and coma.

"In young children, fever might be the only early sign, so parents should see their doctor if concerned, and particularly if their child experiences drowsiness, floppiness, irritability, poor feeding, or general distress."

People most likely to be affected by the Kunjin or MVE virus are newcomers to affected regions, such as babies, young children, tourists or new employees, but anyone experiencing these symptoms should seek medical advice quickly.

"Wet season activity of mosquito-borne viruses now appears to be well underway in the north, with a few reported cases of Ross River and Barmah Forest viruses as well," Dr Lindsay said.

There are no specific cures or vaccines for

Kunjin, MVE, Ross River or Barmah Forest viruses so it is very important that people take care to prevent being bitten by mosquitoes.

The warning particularly applies to people living, visiting or camping near swamp and river systems during the evening and night through the north-east Kimberley and mosquito-prone areas of the Pilbara. However, the viruses may be active elsewhere in the north of the state, especially where mosquitoes are abundant.

"Controlling mosquitoes in most rural regions of WA is generally not possible because of the large size and inaccessibility of natural mosquito breeding habitat," Dr Lindsay said.

People do not need to alter their plans to visit the Kimberley or Pilbara as a result of this warning. However, it is important to avoid mosquito bites by taking a few simple steps, such as:

* avoiding outdoor exposure from dusk and at night in all areas of high mosquito activity;
* wearing protective (long, loose-fitting) clothing when outdoors; and
* using a personal repellent containing diethyl toluamide (DEET) or picaridin. The most effective and long-lasting formulations are lotions & gels. Most natural or organic repellents are not as effective as DEET or picaridin;
* ensuring insect screens are installed and completely mosquito-proof: use mosquito nets and mosquito-proof tents;
* ensuring infants and children are adequately protected against mosquito bites, preferably with suitable clothing, bed nets or other forms of insect screening.

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[Professor John Mackenzie commented in archive no. 20010208.0249: "Kunjin virus is not restricted to Australia. In all, 3 isolates of Kunjin virus were obtained from Sarawak, Borneo in 1966, by Bowen and colleagues (Bowen, et al. Ann Trop Med Parasitol 1970; 64: 263-8).

In addition, Hawkes, Marshall, and our laboratory have found serological evidence of Kunjin virus in Papua New Guinea, and Olson [and colleagues] and Kanamitsu [and colleagues] have found serological evidence of Kunjin virus in parts of the eastern Indonesian archipelago. We have also recently isolated Kunjin virus from mosquitoes trapped on Saibai Island in the Torres Strait, about 4 km from the coast of Western Province of Papua New Guinea. Taken one step further, Kunjin virus has recently been re-classified by the International Committee for the Taxonomy of Viruses (ICTV) as a subtype of West Nile virus. The definition of where Kunjin extends to is not then quite so simple!"

ProMED thanks Lizz Kopecny for sending in the same report.

A map of Australia can be accessed at:
<http://www.map-of-australia.co.uk/map-of-western-australia.htm>
- Mod.TY]

[see also:
2001
Kunjin virus - Australia (NT) (02) 20010208.0249
Kunjin virus - Australia (NT): alert 20010206.0238

.....lm/ty/lm

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# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 2. 16 | 該当なし | |

| 一般的名称 | 乾燥濃縮人血液凝固第Ⅷ因子 | 研究報告の公表状況 | ProMED 20070216-0596, 2007 Feb 16. 情報源:Peru.com, 2007 Feb 16. | 公表国 ペルー |
|---|---|---|---|---|
| 販売名(企業名) | クロスエイトM250(日本赤十字社)<br>クロスエイトM500(日本赤十字社)<br>クロスエイトM1000(日本赤十字社) | | | |

**研究報告の概要**

○黄熱ーペルー(Cuzco)
地方保健局長官は、Cuzco県のLa Convencion郡で黄熱による死亡例が3例発生したと報告した。Cuzcoの保健当局によると、このうち1例はMatoriato地区で発生したとのことである。
当局は、La Convencion郡に向かう人全員を対象とした黄熱のワクチン接種キャンペーンを含む危機管理計画の策定を決定した。

**使用上の注意記載状況・その他参考事項等**

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血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| ペルーのCuzco県La Convencion郡で黄熱による死亡例が3例発生したとの報告である。 | 黄熱ウイルスはウエストナイルウイルスと近縁な脂質膜を持つRNAウイルスである。これまで、本製剤による黄熱ウイルス感染の報告はない。本製剤の製造工程には、平成11年8月30日付医薬発第1047号に沿ったウイルス・プロセスバリデーションによって検証された2つの異なるウイルス除去・不活化工程が含まれていることから、本製剤の安全性は確保されていると考えるが、念のため今後も情報収集に努める。なお、日本赤十字社では帰国後4週間は献血不適とし、輸入感染症の防止に努めている。 |

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**Archive Number** 20070216.0596
**Published Date** 16-FEB-2007
**Subject** PRO/AH/EDR> Yellow fever - Peru (Cuzco)

YELLOW FEVER - PERU (CUZCO)
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail, a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: 16 Feb 2007
From:: ProMED-PORT <Promed-Port@promedmail.org>
Source: Peru.com
[Machine translation edited by Mod.JW]
<http://www.peru.com/noticias/idocs/2007/2/16/DetalleDocumento_382519.asp>

The regional director of Health, Alberto Caro Palavisini, reports that recently 3 deaths have taken place from yellow fever in the province of La Convencion, department of Cuzco.

The regional director of health of Cuzco maintained that one of these deaths was registered in the sector of Matoriato.

The authorities decided to elaborate a contingency plan that includes a mass vaccination campaign against yellow fever, for all people coming to the valleys of La Convencion.

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ProMED
<promed@promedmail.org>

[La Convencion is the northernmost province in the department of Cuzco, beyond Cuzco city & Macchu Picchu. Nevertheless, it would be prudent for travelers to those tourist spots to get vaccinated, to protect against the possibility that a patient harboring the YF virus is hospitalized and infects urban mosquitoes. Macchu Picchu itself is too high for _Aedes aegypti_, but you have to go to Cuzco first in order to get there. See map:
<http://www.nkf-mt.org.uk/images/South%20America/Peru/Cuzco%20District.map.gif
- Mod.JW]

[see also:
2006
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Yellow fever - Peru (Ayacucho): alert 20060318.0836
Yellow fever - Peru 20060101.0002
2004
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Yellow fever - Colombia, Peru 20040314.0710
2003
----
Yellow fever - Peru (San Martin) 20030817.2065
Yellow fever - Brazil, Colombia, Peru 20030219.0432
2001
----
Yellow Fever - Peru (02) 20010607.1120
Yellow Fever - Peru 20010330.0646
1999
----
Yellow Fever - Peru (Junin) 19990610.0991

Yellow fever - Peru (San Martin) 19990509.0762
1995
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Yellow fever - Peru 19950616.0421]
.....jw/lm

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# 医薬品　研究報告　調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年3月2日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Transfusion 47 (1): p162-170 JAN 2007 | 公表国<br>カナダ | |
| 販売名（企業名） | タココンプ（CSL ベーリング株式会社） | | | | | |

**研究報告の概要**

問題点（Simian foamy virus の輸血による感染）

Simian foamy virus (SFV)は非ヒト霊長類レトロウイルスで、非ヒト霊長類を扱う仕事に従事または狩猟するヒトに感染する。

Simian retrovirus の異種間感染は、ヒトでの HIV と HTLV の etiology であると考えられる。SFV は生息宿主では病原性がないが、他の Simian retrovirus と共有系統であるため、異種間感染後に病原性を獲得するか疑問が生じる。

本研究において SFV が輸血により感染する特徴があるか調査した。制御された環境下で、SFV 感染サルの血液が SFV 非感染サルに輸血された。ウエスタンブロット法で、輸血後 9 週目に弱い抗体反応が現れ、12 週目に新生の抗体が強く現れた。

輸血 8 週後に感染したサルの血液から分離した DNA を nested PCR で調べると、proviral DNA が検出された。Real-time PCR で 1000 細胞中に 11 コピーの SFV provirus が測定された。輸血 9 週後では最高値の 39 コピーが測定された。

レシピエント動物の血漿から抽出された核酸を Real-time PCR で調べたところ、検出限界下限レベルの SFV の存在が確認できた。

サルレシピエントの唾液中に感染後 29 週に分子レベルで存在が確認され始めた。慢性的に感染しているサルの唾液中には血漿中で検出される DNA の約 1000 倍のウイルス量が検出された。

本研究が、非ヒト霊長類モデルにおいて輸血による SFV 感染を示す最初の報告であり、全血による SFV 感染がおこることを示すのに説得力がある証拠となる。

示唆的な血漿中の SFV 分子の存在は、血液製剤による SFV 感染リスクに関連する新しい情報を提供するものである。血液供給を守るための手段には、約 $\log 10^3$ の白血球数を減少させる白血球除去が含まれる。細胞関連の病原体の感染性を減弱させるため努力しているが、感染リスクは除去できない。細胞を含有する血液製剤に加え、血漿が SFV 感染源である可能性もあるかもしれない。現在のほとんどの血漿分画製剤は、多種の不活化工程を経るが、原料血漿とプール血漿中にレトロウイルス感染に対する抗体が存在する場合、排除されるべき理由となるであろう。

ヒトからヒトへの SFV 感染の本当のリスクは限られたデータのため、未だに不明である。

使用上の注意記載状況・その他参考事項等

| 報告企業の意見 | 今後の対応 |
|---|---|
| 原料血漿にウイルスが混入する可能性があるが、本剤の製造工程の液状化熱によりレトロウイルスのSFVは不活化できると考えられる。 | 今後ともSFVなど新しい感染症に関する情報収集に努める所存である。 |



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# Characterization of blood-borne transmission of simian foamy virus

*James I. Brooks, Harriet W. Merks, Jocelyn Fournier, Roumiana S. Boneva, and Paul A. Sandstrom*

**BACKGROUND:** Simian foamy virus (SFV) is an endemic, nonhuman primate (NHP) retrovirus that is transmitted to individuals who work with or hunt NHPs. The cross-species transmission of simian retroviruses is believed to be the etiology of human immunodeficiency virus and human T-lymphotropic virus infections in humans. Although SFV is not pathogenic in the native host, the shared ancestry with other simian retroviruses has brought into question the potential for acquired pathogenicity after cross-species transmission. This study examines whether SFV also shares the traits of transmissibility through the blood supply.

**STUDY DESIGN AND METHODS:** Within a controlled environment, blood from an SFV-infected monkey was transfused into an SFV-uninfected monkey. Evidence of infection, pathogenic effects, immune correlates, and viral shedding were followed for 6 months after transfusion.

**RESULTS:** Molecular evidence of SFV infection manifested 8 weeks after transfusion followed by seroconversion 1 week later. Quantitative analysis demonstrated that the highest level of detectable virus was concomitant with seroconversion followed by establishment of a viral "set-point." Analysis of circulating lymphocytes revealed changes early in infection. Potential routes of transmission of SFV and roles of site-specific immune response are suggested by the late appearance of SFV shedding in the saliva of the transfused animal.

**CONCLUSION:** The blood supply has historically provided a portal through which novel, occult viruses can become disseminated among humans. The demonstration of transmissibility of SFV through whole-blood transfusion, in an NHP model, contributes to the understanding of potential risks associated with blood donation by SFV-infected humans.

Simian foamy virus (SFV) is an endemic, nonhuman primate (NHP) retrovirus that is transmissible to humans.[1] Studies have demonstrated that 2 to 3 percent of individuals who work directly with NHPs are persistently infected with SFV.[2,3] In addition, a defined seroprevalence of SFV among bush-meat hunters who harvest NHPs for food has recently been reported.[4] Possibly due to targeted on site blood donor clinics, or due to involvement in biomedical research, we found that the SFV-exposed laboratory workers in Canada donate blood at a much higher rate than the general population (J.I. Brooks et al., unpublished observations). With similar factors promoting blood donation at other primate facilities, it is expected that SFV-exposed workers in general would be overrepresented in the blood donor pool.

The issue of transmission of zoonotic retroviruses through the blood supply is significant, given that human

**ABBREVIATIONS:** CPE = cytopathic effect; LN = lymph node; NHP = nonhuman primate; SFV = simian foamy virus.

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immunodeficiency virus (HIV) and human T-lymphotropic virus (HTLV) infections are both believed to be the result of discrete cross-species transmission events between NHPs and humans.[5] Both of these pathogenic human retroviruses are characterized by prolonged latency before progression to clinical disease. Furthermore, in the case of HTLV, the pathogenic effects of viral infection may only be manifest in a small proportion of infected individuals. Whether SFV shares the pathogenic characteristics of the known human retroviruses after cross-species transmission remains to be determined.

There are relatively few identified human SFV cases, and the period of clinical follow-up remains insufficient for establishing the pathogenic potential of cross-species infection with SFV. Approximately 21 SFV-infected humans have been identified in the published literature with follow-up information available on a few subjects.[1-3,6,7] The available analysis of a subset of infected individuals did not reveal the presence of any medical conditions specifically associated with SFV infection.[1,3] Further clinical information on these infected individuals is being gathered in ongoing follow-up studies. The association of SFV infection with disease is further complicated by an inherent case selection bias in all surveillance programs conducted to date. The recruitment strategy for previous studies captured only SFV-exposed individuals who were relatively healthy and were still working. It is possible that some SFV-infected people, who may have become ill as the result of SFV infection, were no longer working and as a result were not available for studies.

The transmission of the known human retroviruses HIV and HTLV through transfused components of infected blood has resulted in the development of risk management policies specific to populations in whom these infections are prevalent. Since the identification of cross-species transmission of SFV to individuals exposed to NHP, public health agencies in the United States and Canada have attempted to address the risk posed to the blood supply by simian retroviruses.[8,9] A single study reported the lack of SFV transmission to four recipients of blood products from a single SFV-infected donor. The specific circumstances of those transfusions and the small study sample made it difficult to rule out such a risk of SFV transmission.[10] Owing to the absence of SFV transmission events, the potential health consequences from blood-borne transmission of SFV remain unknown. Regulatory authorities have requested more evidence on SFV transmission through blood transfusion to facilitate decision making.

## MATERIALS AND METHODS

### Cynomolgus macaques

A cohort of three, captive-born, cynomolgus macaque monkeys (*Macaca fascicularis*) was selected from the monkey colony at Health Canada. The cohort consisted of an SFV-infected (SFV(+)) "donor" monkey, a blood group matched, SFV-negative (SFV(–)) blood transfusion "recipient," and another SFV(–) "control" monkey that received a saline transfusion. SFV status was confirmed prior to the start of the experiment based upon repeated seroreactivity or seronegativity on an SFV immunoblot and concordant polymerase chain reaction (PCR) results from isolated peripheral blood mononuclear cells (PBMNCs).[2,11]

Three months prior to transfusion, the SFV(–) animals were segregated from the SFV(–) troop, and housed separately. After transfusion, the recipient animal was initially housed in its own room. In order to mitigate the psychological impact of being isolated for 1 month, an SFV(–) companion animal was subsequently brought into the recipient animal's room. The recipient and companion animal were housed singly and could see each other but had no direct contact nor did they share any toys. The control animal was returned to the SFV(–) monkey troop after the saline transfusion. The protocol was approved by the Health Canada Animal Care Committee at the Animal Resource Division. Animals were cared for in accordance with regulations from the Canadian Council on Animal Care.

### ABO blood grouping

Blood group compatibility was determined with reverse typing.[12,13] Serum samples from the donor and eligible recipients were tested for antibodies to A and B antigens with standardized human red blood cells (RBC) reagents (Dominion Biologicals Ltd, Dartmouth, Nova Scotia, Canada). Blood grouping was inferred with Landsteiner's rule from the presence or absence of precipitation to either group A and/or group B RBCs in the presence of serum from the animal in question.

### Cell lines

Canine thymocytes, Cf2Th (ATCC Catalog CRL-1430), and baby hamster kidney cells (BHK21 ATCC Catalog CCl-10) were used to culture SFV-1, -3, and -6, respectively. Cell cultures were maintained according to ATCC recommendations.

### Preparation of SFV antigens

The SFV immunoblot assay for identifying SFV infection in monkeys and humans with a combination of SFV antigens from chimpanzees, African green monkeys, rhesus macaques, and cynomolgus macaques has been described previously.[2,14] Briefly, BHK21 host cells were inoculated with SFV-6, and Cf2Th host cells were inoculated separately with SFV-1 and -3 and SFVmac-cyn. SFV strains were obtained from ATCC (SFV-1, -3, -6) or from our stock

(SFVmac-cyn). At 50 percent cytopathic effect (CPE), the infected cultures of BHK21 and Cf2Th cells were lysed and assayed for protein content. Control cultures of the same cells were lysed at the same time point.

### Preparation of immunoblot

Single-lane NuPage Novex Bis-Tris 4 to 12 percent polyacrylamide IPG gels (Invitrogen, Carlsbad, CA) were loaded with 20 µg of protein from each of the SFV-1, SFV-3, SFV-6, cyn-mac, or 20 µg BHK21 and 60 µg of Cf2Th proteins and run at 200 V for 54 minutes. Three strains of SFV were grown on Cf2Th cells and only one strain on BHK21 cells hence the ratio of proteins loaded on the control gels. Protein was transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Ottawa, Ontario, Canada) with standard techniques.[15] Dilutions of serum (1:100), if available, or plasma were used as the primary antibody. Bound antibody was detected with protein A/G-alkaline phosphatase conjugate (Pierce, Biolynx, Brockville, Ontario, Canada) with chemiluminescent substrate (Immunstar, Bio-Rad, Hercules, CA). Diagnosis of SFV infection was based on seroreactivity to SFV antigens in the 70-kDa range, representing the *gag* doublet, as described previously.[1-3]

### Whole-blood transfusion

The SFV(–) recipient was transfused with 20 mL of fresh blood collected in citrated buffer from the SFV(+) donor animal. This volume represented 10 percent of the circulating blood volume of recipient animal approximating a single unit of whole human blood. The control SFV(–) monkey was treated and housed in the same manner except that it was infused with normal saline. After transfusions, the recipient was isolated as described above and the control animal was returned to an SFV-free colony.

Blood samples from the recipient and control monkeys were collected at 1, 2, 4, 6, 8, 12, and 17 weeks after transfusion for SFV testing. Animals were monitored for serologic and molecular evidence of SFV infection at the indicated time points. Blood was also collected from the recipient monkey at Week 9, to confirm SFV infection and at Weeks 21, 25, 29, and 39 for SFV viral load monitoring.

### Biochemistry and hematologic testing

Anticoagulated whole blood and serum from the recipient monkey was analyzed with commercial assays at a diagnostic veterinary laboratory (Vita-Tech, Markham, Ontario, Canada). The measured parameters included organ systems reported to have disease association with SFV infection.[16] Measured indices included a complete blood count and differential, electrolytes, blood urea nitrogen, liver enzymes, and thyroid function.

### PBMNC isolation

Two milliliters of anticoagulated cynomolgus blood was centrifuged at $1200 \times g$ without brake for 10 minutes. Plasma was carefully removed so as not to disturb the buffy coat. The buffy coat was then removed and layered over 95 percent Ficoll-Paque Plus (Amersham Biosciences, Baie d'Urfe, Quebec, Canada) and centrifuged for 30 minutes, at $1200 \times g$ without brake. PBMNCs were washed twice in RPMI and were cultured immediately or frozen in dimethyl sulfoxide freezing medium (Gibco BRL, Grand Island, NY) and stored in liquid nitrogen.

### Coculture

PBMNCs for coculture were stimulated with 30 U per mL interleukin-2 (IL-2; Roche, Laval, Quebec, Canada) and 5 µg per mL phytohemagglutinin-M (Roche) in RPMI 1640 (Sigma Chemical Co., St. Louis, MO) supplemented in the manner described for Cf2Th cultures with the exception of the addition of 20 percent calf serum for the first 24 hours. Stimulation was typically carried out for a total of 72 hours. Stimulated PBMNCs for coculture were treated with 3000 Rads gamma radiation and $1 \times 10^6$ to $1.5 \times 10^6$ cells were added to a T-25 flask containing $5 \times 10^5$ Cf2Th cells. Cultures were maintained for a minimum of 14 days, split three times per week, and monitored daily for CPE. Stimulated and irradiated control PBMNCs were maintained in culture to monitor for cell death. Uninfected Cf2Th cultures were also maintained to ensure that there was no contamination. After the detection of CPE, SFV infection was confirmed with PCR.

### PBMNC nucleic acid detection

SFV proviral DNA was detected with nested PCR and quantified with real-time PCR. Briefly, DNA was extracted from Ficoll-separated PBMNCs or 2 mL of whole blood with a commercial kit (DNeasy tissue kit or the QIAamp DNA blood midi kit, Qiagen Canada, Mississauga, Ontario, Canada, respectively). The choice of whole blood or PBMNCs as source material was based on sample availability. Nested PCRs were performed on a thermocycler (ABI 9700, Applied Biosystems Canada, Streetsville, Ontario, Canada) as previously described[11] with the exception of the elimination of the initial two cycles at 38°C. Presence of a 464-bp band on an ethidium bromide–stained agarose gel was deemed a positive. Identity of this band was confirmed, when first appearing in a sample, by DNA sequencing.

Real-time PCR primers were optimized for SFVmac-cyn using computer software (Primer Express, Applied Biosystems) with the target located within the same con-

served area of the *pol* gene. Cycling conditions included denaturation for 15 seconds at 94°C and anneal-extend for 1 minute at 60°C for 50 cycles after following the manufacturer's recommendations for UNG and *Taq* activation (TaqMan universal PCR master mix, Applied Biosystems). Primers and probe were as follows: sense MS5 5′-ACCGGACGAGATGCTACCTTT, antisense MS6 5′-TGA CTTTTACCACATCCTTTCGTAGA, and probe 6-FAM-TGAAAGTCTCTTCCAAGTATTGGTGGCCAA-TAMRA. The standard curve consisted of serial dilutions of a cloned fragment of the *pol* gene SFVmac-cyn in a background of SFV(–) *M. fascicularis* genomic DNA. The assay could reliably detect 10 copies of target DNA in a background of genomic DNA. Real-time PCR procedures were carried out on a sequence detection system (ABI Prism 7000, Applied Biosystems) with 1 µg of target DNA. The effective number of cells added to the quantitative assay was calculated by dividing the amount of DNA added to the reaction by the mass of DNA in a diploid *M. fascicularis* cell.[17] Quantification of SFV viral load is represented as the number of copies per 1000 cells.

### Plasma and saliva nucleic acid detection

Total nucleic acid was recovered from 200 µL of plasma or saliva with a virus kit (QIAamp Ultras-Sens, Qiagen) in a volume of 40 µL. Samples were used neat or DNase treated on the column according to the manufacturer's instructions (Qiagen). At Week 39, plasma samples were treated as above with and without passage through a 0.45-µm filter used to eliminate cellular material. For all samples, 10 µL of the extracted material was reverse transcribed and amplified with the a probe reverse transcription (RT)-PCR kit (QuantiTect, Qiagen) according to manufacturer's directions with the MS5 and MS6 primers and Taqman probe with the conditions described above with an initial 30-minute (reverse transcriptaise) incubation at 50°C. Plasmid DNA containing SFV pol sequence, as described above, was used as a positive control.

### Analysis of lymph node from recipient monkey

At 17 weeks after transfusion, a left inguinal lymph node (LN) was harvested under general anesthesia from the recipient monkey. The LN was divided in half and real-time PCR was carried out on one portion of the LN after nucleic acid extraction with a tissue kit (DNeasy, Qiagen). The other portion of the LN was teased apart and filtered through a 100-µm cell strainer, and cells were separated with a Ficoll gradient. The purified lymphocytes were then divided into two aliquots. One portion of cells was stimulated as described above, irradiated, and cocultured with Cf2Th until CPE was seen at which point cellular material was analyzed with real-time PCR. The other portion of the purified cells was stimulated as above for 3 days and then cultured for 7 additional days in the presence of IL-2. After 10 days' total culture, these latter cells were harvested and washed. RNA was isolated with an RNA blood mini kit (Qiagen) and treated with the RNase-Free DNase set (Qiagen). Sample was reverse-transcribed and PCR-amplified with a one-step RT-PCR kit (OneStep, Qiagen) according to the manufacturer's directions with primer-3 and primer-4 described previously.[11]

### Sequence analysis

Sequencing was performed on a capillary sequencer (ABI 3100, Applied Biosystems), with dye terminator chemistry (Big Dye v.3, Applied Biosystems), according to the manufacturer's instructions with primers 1, 2, 3, or 4.[11]

### Flow cytometric analysis

The samples were analyzed with a flow cytometer (FACS-Calibur, BD Biosciences, San Jose, CA) equipped with two lasers: an argon-ion laser (488 nm) and a red diode laser (635 nm). Anti-human monoclonal antibodies CD45, 3, 4, 8, 20, and 16/56 (BD Biosciences, Mississauga, Ontario, Canada) were used for four-color immunophenotyping on fresh ethylenediaminetetraacetate blood.[18] To determine absolute and percentage values with single-platform technology, fluorescent particles (AccuCount Spherotech, Libertyville, IL) were used as internal calibrator beads. Both lymphocyte subset percentage and absolute count enumeration was generated with a CD45-based gating strategy.

## RESULTS

### Pretransfusion analysis

Initial experiments confirmed that RBC ABO epitopes were not present in sufficient quantity to be detected by forward grouping.[13] Instead, the presence of antibodies to A and B antigens in both the donor and the recipient monkey established a common type O blood group (data not shown).

The donor animal had serologic evidence of SFV infection at all time points tested before transfusion (Fig. 1) and was demonstrated to propagate infectious virus by the production of characteristic CPE after 10 days of coculture of donor monkey PBMNCs with Cf2Th (data not shown). The recipient animal had been identified as SFV(–) 2 years previously and remained seronegative at all points before transfusion (Fig. 1).

### Demonstration of seroconversion after transfusion of SFV-positive blood

Consistent with the transfer of passive immunity, 1 week after the transfusion, the recipient monkey showed weak seroreactivity to SFV gag antigens on the immunoblot

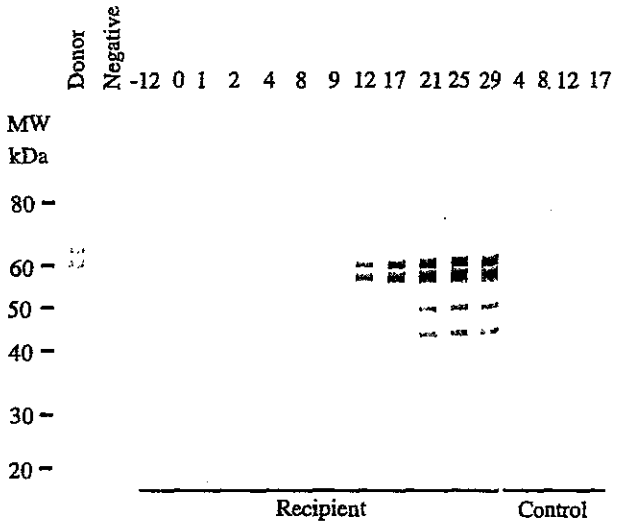


**Fig. 1. Seroreactivity of transfused monkey plasma over time (transfusion time points in weeks) toward lysates of SFV-1, SFV-3, SFV-6, and $SFV_{Mac}$ with Western blotting. Negative control serum from cynomolgus macaque.**

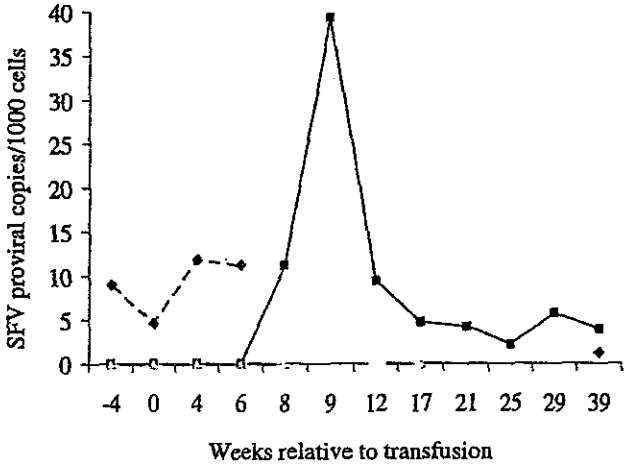


**Fig. 2. SFV proviral load. (■) Recipient; (▲) control; (◆) donor.**

assay that disappeared by Week 8. On the original immunoblot, a weak antibody response is manifest at 9 weeks after transfusion. At Week 12, however, a robust de novo antibody response is easily appreciated, with increasing antibody titer as demonstrated through the increase in intensity banding at successive sampling times (Fig. 1). The control monkey did not display any serologic evidence of SFV infection through 17 weeks after saline transfusion.

## Molecular evidence of transfusion acquired SFV infection

Proviral DNA was detected by nested PCR in DNA isolated from the blood of the transfused monkey 8 weeks after transfusion heralding seroconversion that manifested 1 week later (Fig. 2). Before this, all time points were negative for proviral DNA in the recipient animal. Subsequent analysis by real-time PCR revealed 11 copies of SFV provirus per 1000 cells at this time point (Fig. 2). The highest cellular viral load measured was 39 copies per 1000 cells at Week 9. Subsequent SFV viral load measurement revealed a "set-point" cellular viral load in the recipient monkey of 3 to 4 SFV proviral copies per 1000 cells. These numbers compare with the donor monkey SFV viral load of 13 copies of SFV per 1000 cells. No PCR evidence of SFV infection could be found in the control monkey at any time point (Fig. 2).

## Evidence of SFV in plasma

Real-time RT-PCR analysis of total nucleic acid extracted from the recipient animal's plasma showed the presence of SFV at the lower limit of detection. Positive signal was present on replicate testing at the aggregate time point of 8 and 9 weeks and at Week 25 (Table 1). The positive SFV signal detected in the plasma of the recipient monkey disappeared after DNase treatment. In a similar fashion, the donor animal had SFV detected in the plasma at Week 0, but again this signal disappeared after DNase treatment. The control animal had no positive results with this assay.

**TABLE 1. Detection of SFV nucleic acid in plasma with real-time RT-PCR***

| Sample | Time point (weeks) | Positive results/ replicate number |
|---|---|---|
| Donor | 0 | 1/4 |
| Donor | 39 | 0/1 |
| Recipient | 0 | 0/1 |
| Recipient | 4 | 0/4 |
| Recipient | 8 | 2/4 |
| Recipient | 9 | 3/4 |
| Recipient | 12 | 1/4 |
| Recipient | 17 | 0/4 |
| Recipient | 21 | 0/1 |
| Recipient | 25 | 1/2 |
| Recipient | 29 | 0/1 |
| Recipient | 39 | 0/1 |

* All positive results became negative after DNase treatment. No SFV was detected from the plasma of the control monkey at any time point.

## Viral shedding in saliva

The recipient monkey showed molecular evidence of viral shedding in saliva beginning at 29 weeks after infection (Table 2). At 39 weeks after infection, both viral RNA and DNA were detected in saliva of the recipient monkey. The donor monkey's saliva was positive for viral DNA and RNA at all tested time points (Table 2). The quantity of virus detected in the saliva of the chronically infected monkey

was approximately 1000-fold higher than that detected in the plasma.

**TABLE 2. Detection of SFV nucleic acid in saliva with real-time RT-PCR***

| Sample | Time point (weeks) | Total nucleic acid | After DNase treatment |
|---|---|---|---|
| Donor | 6, 29, 39 | + | + |
| Recipient | 6-21 | – | ND† |
| Recipient | 29 | +‡ | – |
| Recipient | 39 | + | + |

* No SFV was detected in the saliva of the control monkey.
† ND = not determined.
‡ Positive in one of two replicates.

### LN analysis after transfusion

DNA extracted from inguinal LN tissue, at week 17, contained 4.2 copies SFV proviral DNA/1000 cells by Real-Time PCR analysis (data not shown). Characteristic CPE of SFV infection was seen after 14 days of co-culture with stimulated lymphocytes isolated form the lymph node. Evidence of SFV nucleic acid was present in whole nucleic acid extracts using Real-Time PCR. Nucleic acid extracts of isolated and stimulated lymphocytes demonstrated evidence of SFV by Real Time PCR that persisted after DNase treatment.

### Biochemistry, hematologic markers, and clinical changes

Clinical laboratory measures showed little change over the course of the study. White blood cell (WBC) counts differed among animals but remained consistent throughout the experiment. There was no evidence of a hepatitis induced by infection. Thyroid function as measured by the free T4 assay was not affected by infection.

### Flow cytometry results

The total lymphocyte count on both the transfusion and the control animals fell after the transfusion consistent with a stress response brought on by housing in isolation (Fig. 3). The CD4+:CD8+ ratio remained fairly constant for the control animal with minor variations over time. At Week 4 after transfusion, however, the recipient animal had a sudden inversion in CD4+:CD8+ ratio from 1.2 to 0.8 (Fig. 4). The ratio of CD4+:CD8+ remained in flux with a value of 1.8 measured at Week 6 appearing to stabilize beyond Week 12.

The change in the CD4+:CD8+ ratio appears due to a precipitous drop in the percentage of CD4+ cells beginning at Week 2 after transfusion, continuing to a nadir at Week 4 with recovery by Week 6 (Fig. 5). With a delay of approximately 2 weeks, the percentage CD8+ cells also showed a precipitous drop contributing to the large rebound in the CD4+:CD8+ ratio. By 12 weeks, the percentages appear to stabilize, but the animal demonstrates a higher relative contribution of CD8+ cells than at baseline.

### Sequencing results

Comparison of a 425-bp sequence within the *pol* gene of SFV isolated from the donor and recipient monkeys showed 99.6 percent homology (Fig. 6).

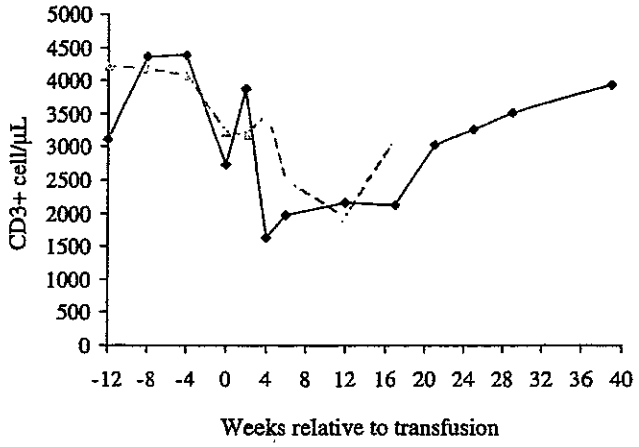


**Fig. 3. Total T-lymphocyte count. (◆) Recipient; (▲) control.**

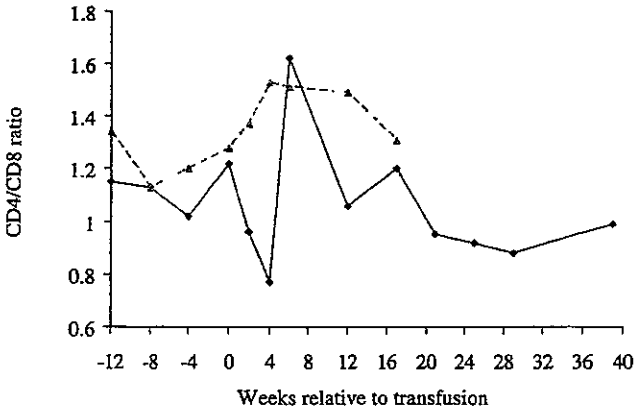


**Fig. 4. Changes in CD4/CD8 ratio. (◆) Recipient; (▲) placebo.**

## DISCUSSION

To our knowledge, this is the first report showing transmission of SFV via blood transfusion in an NHP model. Previous studies demonstrating transmission of SFV have been performed in small animals with techniques such as intradermal inoculation to demonstrate the presence of an infectious agent.[19] By employing stringent experimental conditions to ensure that the recipient animal acquired SFV infection by means of the blood transfusion and not through any other route, we provide compelling evidence

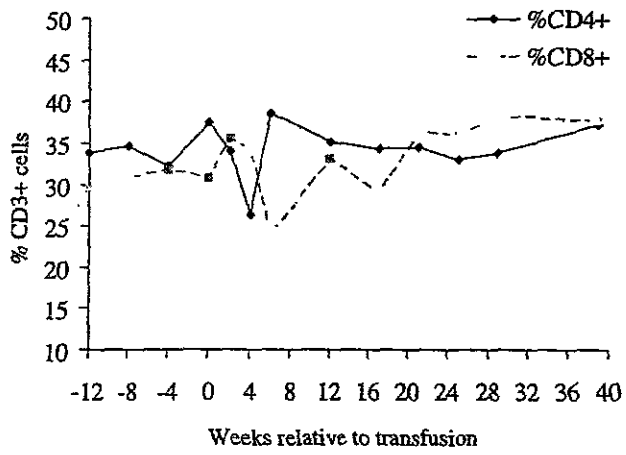


**Fig. 5. Relative percentages of lymphocytes in recipient. (◆) Percent CD4+; (■) percent CD8+.**

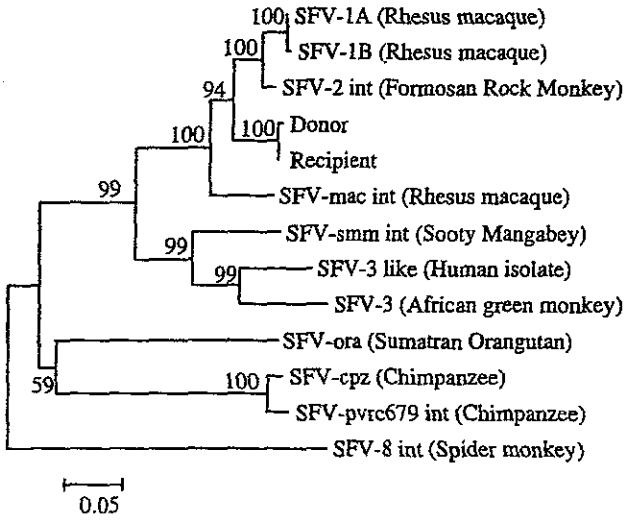


**Fig. 6. Phylogenetic relationship of integrase sequences of SFV-infected recipient and donor monkey compared with other primates. The tree was derived by NJ analysis with two Kimura distances. Values on branch nodes represent the percentages of 1000 bootstrap replicates. The scale bar represents evolutionary distance of 0.05 nucleotide per site.**

for the blood-borne transmission of SFV with whole blood. This finding suggests that SFV may be transmissible to humans through SFV-contaminated blood. In our previous study,[2] half of 46 animal handlers reported a history of blood donations (J. Brooks, unpublished data). This figure compares with 2 percent of the general population that donate blood on a regular basis,[20] suggesting that animal handlers may be overrepresented in the donor pool. Given this new knowledge, the question on the deferral of potentially SFV-infected persons remains open for debate.

This study also provides the first detailed information on acute SFV infection in the native host. Similar to what has been described for other retroviruses, SFV infection appears to result in a burst of viral replication leading to a spike of circulating virus which then falls to achieve equilibrium of viral set-point after seroconversion. Although the containment of the rapid increase in SFV replication is most likely the consequence of intervention by the immune system, the exact role of humoral and cellular immunity remains to be elucidated. Clearly, there is an increasing titer of specific antibodies against SFV that may be neutralizing as seen in other models.[21] However, the CD4+:CD8+ inversion, in the recipient and not the control monkey, in the 2 to 4 weeks after the transfusion, suggests stimulation of a cell-mediated immune response to the infection. Another possibility is that there may be either viral- or immune-mediated killing of SFV-infected T-cell targets because previous work has shown that lymphocytes are the major reservoir of SFV in the blood of infected animals.[22] Other explanations for the T-cell disturbances could include the nonspecific allogeneic effects of the blood transfusion or cotransmission of an occult pathogen. There was a parallel drop in the total CD3+ in both the transfused and the control monkey in the 4 weeks after the transfusion. This effect is likely due to stress associated with the single housing arrangements.[23]

Analysis of the excised LN indicates that the potentially infectious virus is found in the lymphatics. The LN harvested at 17 weeks had SFV DNA detectable at the time of excision. When stimulated with IL-2 and phytohemagglutinin, these cells then produced a highly transcriptionally active virus indicated by detection of viral RNA from lysed cells.

We also describe the seeding and emergence of active SFV replication in saliva from the recipient animal. SFV DNA is initially detected in saliva followed by the emergence of viral RNA concomitant with the development of SFV-specific immunity and the reduction in measurable virus within the circulating lymphocytes. This finding may suggest a qualitative difference in the capacity of the host immune system to suppress viral replication in the two sites. The presence of SFV in the saliva supports the possibility that the virus may be transmitted through bites.[24] The delay in the appearance of SFV in the saliva suggests that a recently infected animal may not be able to transmit the virus through saliva, until 6 or more months after infection.

The data on isolation of SFV from the oropharynx of infected humans is less clear. In the largest study, SFV was successfully cultured from a throat swab in only one of six SFV-infected individuals but isolation from the saliva was unsuccessful in all cases.[25] Molecular evidence of SFV in the saliva was detected only in that one individual who had the positive throat culture. Another report in the literature describes failure to culture SFV from saliva in a single individual.[7]

The demonstration of viral DNA in the plasma may represent viremia. It could be argued that our results represent contamination from cellular DNA. Our preparative methods, however, are consistent with those used to obtain HIV nucleic acid for viral load testing. Virus in the plasma in association with the peak viral load in cellular DNA is consistent with maximum viral turnover, perhaps at other sites such as the lymphatics, with spillover of free virus into the blood compartment. The detection of viral DNA in the plasma, in association with peak viremia, is not surprising given our understanding that the DNA form is the functionally relevant virus.[26,27] SFV replication is unique among retroviruses as it is reverse-transcribed late in its life cycle,[26] and SFV has been described as a DNA virus with an RNA intermediate.[27] Our detection of RNA forms of SFV from the cell culture may represent the cell-associated intermediate forms of SFV associated with the viral life cycle.

Suggestive molecular evidence of SFV in the plasma provides new information relevant to the risk of SFV transmission through blood products. Measures to protect the blood supply include leukoreduction, which reduces the total number of WBCs by approximately 3 logs.[28] Although this effort has been shown to reduce infectivity of cell-associated agents,[29] risk of infection is not eliminated. In addition to blood products containing cells, the possibility now exists that plasma may be a source of transmissible SFV. Although most current plasma products undergo a variety of inactivation processes, the presence of antibodies to infectious retroviruses in source plasma and plasma pools could be considered grounds for donor exclusion.[29]

The risk of SFV becoming endemic in the human population depends on the prevalence of SFV infection, the transmissibility of the virus, and the opportunities for human-to-human transmission. There is a measurable prevalence of infection and ongoing transmission of SFV to animal workers and people who hunt NHPs. Studies have shown that 2 to 3 percent of people who work with NHPs are infected with SFV.[1,2] The majority of captive NHPs are SFV-infected.[30] Previously, we have demonstrated human SFV infection resulting from cross-species transmission of the virus from macaques, the most common NHP used in the laboratory setting.[2] This study establishes that SFV is transmissible through blood transfusion with a homologous virus challenge in the native host. Taken together, these findings indicate that there may be a risk of SFV transmission through the blood supply from individuals occupationally exposed to NHPs.

The true risk of human-to-human transmission of SFV remains unknown due to limited data. The only published study on blood donation by an SFV-infected human failed to demonstrate transmission to four recipients of blood products.[10] The largest collection of data examining the risk of transmission of SFV between humans is limited to six spouses of SFV-infected men where transmission was not observed.[3] The absence of transmission from a small number of sexual partners of SFV-infected men, however, does not rule out transmissibility of SFV between humans via other risk behaviors. Furthermore, in the context of the blood supply, retroviral transmission risk may be 3 orders of magnitude higher than through unprotected sexual contact.[31,32]

In principle, dissemination of a novel retrovirus into the human population should be prevented. The blood supply has historically provided a portal through which novel occult viruses can become disseminated among humans. Data provided by this study may guide decisions regarding blood donor deferral of people with ongoing exposure to NHPs, to minimize the risk of transmission of SFV through blood transfusions. These decisions will have to be weighed against negative health effects, including blood availability.[33]

### NOTE

Subsequent to submission of this work, similar findings were published by Khan et al. in Transfusion 2006;46:1352-9.

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# 医薬品　研究報告 調査報告書

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**研究報告の概要**

輸血によるウエスト・ナイル・ウイルス(WNV)感染が初めて報告されたのは2002年であり、2003年までに供血の全国的なWNV検査が迅速に導入された。スクリーニングは6又は16人のミニプール核酸増幅検査(MP-NAT)が用いられ、後に個別の供血NAT(ID-NAT)による強化スクリーニングが、WNV流行時期にMP-NAT陽性数が既知のトリガー閾値に達した場合(連続7日間で500件に1件以上の陽性が検出された場合)に実施された。
WNV輸血感染患者は、スクリーニング実施前の2002年は23名、2003年は6名、2004年は1名、2005年は0名であった。
本報告はID-NATを使用した強化スクリーニングが開始されて以降、初めて2006年に2名の免疫抑制状態の患者がウエスト・ナイル神経侵襲性疾患(WNND)を発症したとの報告である。
一例は腎移植を受けた末期腎疾患の82歳の男性で、発症の8週間前に6名の供血者由来の血液成分を投与されており、その内1本の濃厚赤血球の供血者がIgM抗体陽性であった。
供血者の識別後、その血液からの血小板および新鮮凍結血漿(FFP)が遡って追跡調査された結果、さらに腎移植を5年前に受けていた60歳の男性にFFPが投与されており、この男性もWNV関連脊髄炎を発症し、脳脊髄液(CSF)中にIgM型の抗WNV抗体が検出された。
WNNDの発症率はWNV感染症全体(大半は蚊由来)の1%未満であるが、移植患者のWNND発症リスクは一般集団の4倍以上と推定される。
今回の供血から遡って1ヵ月の間に実施された血液採取期間の検査では、MP-NAT陽性結果が2件検出されていたが、この2件の陽性結果の間には7日間以上の開きがあったため、MP-NATは実施されたがトリガーID-NAT検査は実施されなかった。今回の調査結果は、偽陰性MP-NAT結果が生じる可能性のほか、トリガーID-NATに関する戦略を検討する必要性を示唆している。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 輸血用血液成分製剤によるWNV感染例の報告であるが、血漿分画製剤でのWNV感染伝播の報告はなく、製造工程中にWNVと同じフラビウイルスであるウシ下痢症ウイルス(BVDV)の不活化除去が確認された工程を設けているが、今後とも関連情報に注意していく。 | 今後ともWNVに関連する情報の収集に努めていく。 |

**使用上の注意記載状況・その他参考事項等**

添付文書の冒頭部分
原料となった血液を採取する際には、問診、感染症関連の検査を実施するとともに、製造工程において一定の不活化・除去処理などを実施し、感染症に対する安全対策を講じているが、ヒト血液を原料としていることによる感染症伝播のリスクを完全に排除することはできないため、疾病の治療上の必要性を十分に検討の上、必要最小限の使用にとどめること。

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Since mid-January, RVF in livestock has been detected in districts surrounding Nairobi, signaling occurrence of the outbreak in new areas. Reports also have been received of livestock and humans with illness consistent with RVF across the border in Somalia, where disease assessment has been hampered by ongoing security concerns. Several international organizations are collaborating to control the spread of the outbreak within Kenya and to other countries. Travelers should take precautions when visiting RVF-affected areas. Generally, the risk for RVF infection among travelers to Kenya is low, unless they visit areas where an outbreak is occurring and are bitten by infected mosquitoes or come in contact with body fluids, uncooked tissue, or aerosols from infected livestock. No preventive RVF medications or licensed vaccines for humans exist. Travelers to affected areas should reduce their risk for infection by protecting themselves from mosquito bites and by avoiding direct contact with livestock. Specific recommendations for U.S. travelers are available at http://www.cdc.gov/travel/other/2006/rift_valley_fever_kenya.htm.

To control the outbreak, KMOH launched several interventions, some of which might have limited the public health impact of the outbreak. A ban on the slaughter of animals (including during Eid-ul-azha, a religious holiday) was imposed in NEP and strictly enforced. The Ministry of Livestock and Fisheries Development initiated a policy of vaccinating apparently unaffected herds of livestock in districts in which human or livestock RVF disease had been confirmed and also in adjacent districts; however, as of January 25, only a small proportion of livestock had been immunized. Other interventions included heightened disease surveillance among humans and animals, community mobilization, animal quarantines and restricted transport of livestock, and an integrated vector-control strategy, including indoor residual spraying and larviciding. RVF wards were established in which appropriate infection-control measures were encouraged.

Timely detection of this outbreak was aided by implementation of Integrated Disease Surveillance and Response* within most of the affected districts. A second factor contributing to timely detection was initiation of RVF laboratory-supported field surveillance of febrile patients at outpatient clinics in Garissa. Ongoing epidemiologic, entomologic, and veterinary studies related to this outbreak continue to 1) identify factors associated with severe forms of RVF illness and poor outcomes; 2) characterize the role of specific species of mosquitoes in transmitting, maintaining, and spreading RVF virus; 3) assess the economic impact of the outbreak; and 4) define the impact of livestock immunization with live, attenuated RVF veterinary vaccine on minimizing the spread of animal and human disease. Taking measures to decrease contact with mosquitoes through use of repellents and bednets and avoiding exposure to blood or tissues of animals that might be infected are important protective measures for preventing RVF. Livestock vaccination also can be an effective means of preventing cases of human RVF if adequate vaccination coverage and herd immunity are achieved.

*A strategy of the African Regional Office of WHO that aims to improve availability and use of surveillance and laboratory data to control infectious diseases that are the leading causes of death, disability, and illness in the region.

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## West Nile Virus Transmission Through Blood Transfusion — South Dakota, 2006

West Nile virus (WNV) transmission through blood transfusion was first reported in 2002 (*1,2*), prompting rapid implementation of nationwide screening of blood donations for WNV by 2003 (*3,4*). Screening strategies were developed using minipool nucleic acid-amplification testing (MP-NAT) based on six or 16 pooled donor samples. To improve sensitivity of WNV detection, blood-collection agencies (BCAs) later implemented enhanced screening by individual donation NAT (ID-NAT), most often used when a given trigger threshold of positive MP-NAT results is reached during the WNV transmission season (*5,6*). This approach has been effective, resulting in the detection and interdiction of

approximately 1,400 potentially infectious blood donations during 2003–2005 and a reduction in recognized transfusion-transmission events (*7*). A total of 23 confirmed WNV transfusion-transmitted cases were reported in 2002, before screening was implemented; six probable or confirmed cases were detected in 2003 after MP-NAT screening was initiated, one was detected in 2004, and none were detected in 2005 (*7*). This report describes the first WNV transfusion-transmission cases detected since the initiation of enhanced screening strategies using ID-NAT triggering. In 2006, two immunosuppressed patients had onset of West Nile neuroinvasive disease (WNND) after receiving blood products from a single infected donor despite a negative MP-NAT result at the time of donation. Although risk for transmission has been substantially reduced as a result of routine MP-NAT and triggered ID-NAT screening, clinicians should be reminded that transfusion-transmitted WNV infections can still occur, and that immunosuppressed patients are more likely to have onset of WNND.

In September 2006, the South Dakota Department of Health (SDDH) was notified of WNND in a man aged 82 years with end-stage renal disease who had received a kidney transplant on August 25, 2006. Four days after the transplant surgery, the patient received a transfusion of 2 units of packed red blood cells (PRBC) for anemia. Ten days after surgery, the patient was discharged to a long-term–care facility and continued to receive immunosuppressive therapy, including 750 mg of mycophenolate mofetil twice daily, 125 mg of cyclosporin twice daily, and 20 mg of prednisone daily. Twenty-one days after surgery, he had onset of fever, lethargy, and a peri-incisional hematoma, prompting his readmission to the hospital. The patient was treated empirically with broad-spectrum antimicrobial and antifungal agents. Two days after readmission (i.e., 23 days after transplant and 19 days after PRBC transfusion), his mental status deteriorated rapidly. The next day, his cerebrospinal fluid (CSF) had four white blood cells (WBC)/$mm^3$, 46 red blood cells (RBC)/$mm^3$; a protein level of 58 mg/dL, and a glucose level of 67 mg/dL. Anti-WNV immunoglobulin M (IgM) antibody was detected in both serum and CSF by IgM antibody-capture enzyme-linked immunosorbent assay (MAC-ELISA) performed at SDDH. When the patient was discharged to a long-term–care facility (36 days after his transplant surgery), his fever had resolved, and his mental status had improved.

Because the patient had been hospitalized during the 2 weeks before onset of his WNV-related illness, WNV transmission by organ transplantation or blood transfusion was considered more likely than transmission by mosquito bite. The kidney donor's premortem serum was negative for both anti-WNV IgM and WNV RNA by MAC-ELISA and reverse transcription polymerase chain reaction (RT-PCR). One other kidney transplant recipient from the same organ donor had no symptoms of WNV disease, and serum from this recipient was negative for both anti-WNV IgM and WNV RNA. Traceback investigation revealed that the patient with WNND had received blood products from six different donors during the 8 weeks before symptom onset. No donor samples from the time of donation were available for testing. However, all donors consented to have serum collected and tested for anti-WNV IgM. One donor, the source of 1 PRBC unit transfused into the patient with WNND 4 days after transplant, was IgM positive.

The implicated blood donor was a man from a rural area of South Dakota where substantial WNV activity in birds, mosquitoes, and humans occurred during the 2006 transmission season. He had not traveled outside of South Dakota during the month before his last donation on August 4, 2006. He did not report any symptoms consistent with WNV disease during the 2 weeks before this donation or during the 3 subsequent months. Because the BCA that collected the donation did not conduct routine screening for WNV, a sample of the donor's blood was sent for screening at an out-of-state BCA, where the MP-NAT test result for six pooled samples, including his donation, was negative. The out-of-state BCA had a policy of triggering ID-NAT after two WNV-positive MP-NAT results and more than one positive in 500 results during a rolling 7-day period. Two positive MP-NAT results had been detected by the testing BCA during the month before this donation; however, the positive results occurred more than 7 days apart and therefore did not trigger ID-NAT testing.

After identification of the IgM-positive donor, the platelet and fresh frozen plasma (FFP) co-components from his whole blood donation were traced. The platelet unit had been discarded without being transfused. The FFP unit had been transfused on August 10, 2006, into a man aged 60 years who had received a kidney transplant in 2001 for end-stage renal disease attributed to insulin-dependent diabetes mellitus. On the same day as the transfusion, he had undergone surgical repair of a spinal fracture caused by a fall. He received a transfusion of 15 blood products, including 6 units of FFP, one of which was from the blood donor described in this report. One week after surgery, he was discharged to a rehabilitation facility, where he continued to receive immunosuppressive therapy, including 4 mg tacrolimus twice daily and 500 mg mycophenolate mofetil three times daily. Eleven days after the surgery, he had onset of fever and was treated empirically with antimicrobial and antifungal agents. Fifteen days after surgery, he had onset of tremors, encephalopathy, and acute left arm paralysis unexplained by his previous injury but

consistent with WNV-associated myelitis. The patient's CSF had four WBC/mm$^3$, zero RBC/mm$^3$, a protein level of 171 mg/dL, and a glucose level of 52 mg/dL. Anti-WNV IgM was detected in the CSF by MAC-ELISA at SDDH. The patient's fever, tremors, and encephalopathy resolved, but his left arm paralysis persisted at the time of transfer to an out-of-state hospital 5 days after symptom onset (20 days after surgery). Three months later, the patient remained in a long-term–care facility.

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**Editorial Note:** This report describes two cases of probable transfusion-transmitted WNV from a common blood donor despite a negative MP-NAT result at the time of donation. The source of infection cannot be proven because blood samples or co-components from the implicated donation were unavailable for testing; however, evidence of WNND in two recipients of blood products from a common donor with serologic evidence of recent infection makes WNV transfusion-transmission probable. Because these two transfusion recipients were hospitalized for at least 2 weeks each before onset of WNND, neither patient was likely to have acquired infection from a mosquito bite. Furthermore, for the patient who underwent transplant surgery on August 25, transmission through the transplanted kidney is unlikely, given that neither the organ donor nor the other organ recipient had evidence of WNV infection.

Nationwide blood screening for WNV has been successful in preventing transfusion-transmitted WNV (*3*). However, as with all blood donation screening, infections can be transmitted to transfusion recipients on rare occasions despite negative donor test results. Although WNV transmission by blood transfusion is rare, the cases described in this report underscore the importance of clinical recognition, effective WNV blood screening strategies, and investigation coordination.

Transfusion-transmitted WNND might be difficult to recognize, but physicians should consider the disease as a possible diagnosis, particularly when unexplained neurologic complications occur in immunosuppressed patients after transfusion. Both patients described in this report were kidney transplant recipients who were immunosuppressed when they had onset of WNND after receiving blood product transfusions. Although WNND occurs in less than 1% of WNV infections overall (the majority of which are mosquito-borne), transplant patients who acquire WNV infections have an estimated forty-fold greater risk for developing WNND compared with the general population (*8*).

The results of this investigation highlight the potential for false-negative MP-NAT results and the need to evaluate strategies for triggering ID-NAT donor screening; however, they also underscore the rarity of WNV transfusion-transmission events. Since ID-NAT triggering was fully implemented after the start of the 2004 transmission season, no transfusion-transmitted cases had been detected until the cases described in this report. Most false-negative MP-NAT results are caused by low-level viremic donor samples in which WNV is undetected by MP-NAT but is potentially identifiable by the more sensitive ID-NAT. Criteria for triggering ID-NAT differ among BCAs, but most are based on the number of positive MP-NAT results or a threshold rate for all positive results reached during a rolling 7-day period (*5*). Certain BCAs collect blood and perform NAT screening on-site; however, BCAs without the ability to screen for WNV send donor samples to remote (sometimes out-of-state) BCAs for testing. BCAs performing the testing determine when to trigger ID-NAT upon reviewing their own results.

To enhance the sensitivity of ID-NAT triggering, BCAs are considering the feasibility and utility of more standardized criteria for ID-NAT triggering and methods for enhanced communication among BCAs so that knowledge of positive screening results can be shared. BCAs face many challenges in WNV screening, including seasonal epidemics that are geographically unpredictable, limited resources for ID-NAT, and coordination of blood collection and testing that might be performed by multiple BCAs in a given geographic area. An additional tool for sharing of donor screening results might be useful to enhance ID-NAT triggering. The WNV Biovigilance Network,* currently being piloted by AABB (formerly known as the American Association of Blood Banks) to aggregate WNV blood donor screening results, is a model for successful collaboration. However, timeliness of reporting must be addressed to adapt the network for use in decisions regarding ID-NAT triggering.

Public health investigations involving patients with recent transplantation or blood transfusion are complex and often involve multiple states and local jurisdictions. Coordination among state and local health departments, clinicians, BCAs, hospital blood banks, transplant centers, and CDC often is required. Prompt reporting of suspected cases to local and state health departments, with assistance from CDC, will promote timely traceback investigations that can identify additional cases and prevent further transmission.

* Information available at http://www.aabb.org/content/programs_and_services/west_nile_virus_study/wnvstudy.htm.

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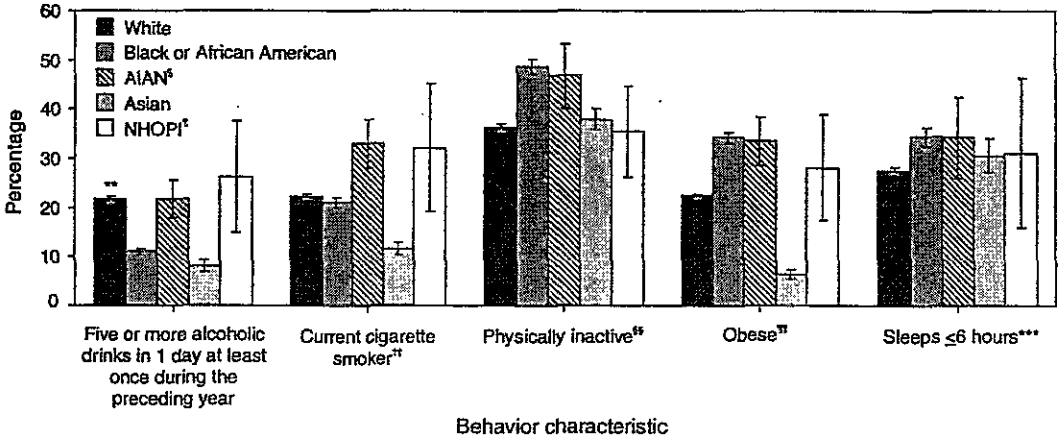
# QuickStats

**FROM THE NATIONAL CENTER FOR HEALTH STATISTICS**

## Prevalence of Selected Unhealthy Behavior Characteristics Among Adults Aged ≥18 Years, by Race* — National Health Interview Survey, United States, 2002–2004†




* Racial categories include persons who indicated a single race only and are consistent with the 1997 Office of Management and Budget federal guidelines for race reporting.
† Estimates are age adjusted using the 2000 projected U.S. population as the standard population and using three age groups: 18–44 years, 45–64 years, and ≥65 years. Estimates are based on household interviews of a sample of the civilian, noninstitutionalized U.S. adult population. Denominators for each percentage exclude persons with unknown health-behavior characteristics.
§ American Indian or Alaska Native.
¶ Native Hawaiian or other Pacific Islander.
** 95% confidence interval.
†† Smoked at least 100 cigarettes in lifetime and currently smoked.
§§ Never engaged in any light, moderate, or vigorous leisure-time physical activity.
¶¶ Defined as a body mass index (weight [kg]/height [$m^2$]) of ≥30.
*** Usual number of hours of sleep during a 24-hour period. Based on data from 2004 only.

The percentage of adults with selected unhealthy behavior characteristics varied by race during 2002–2004. Blacks and Asians had the lowest prevalence of consuming five or more alcoholic drinks in a single day; Asians also had the lowest prevalence of current cigarette smoking and obesity. AIAN had among the highest prevalences of consuming five or more drinks, current smoking, and obesity. Generally, physical inactivity was the most prevalent unhealthy behavior.

**SOURCE:** Adams PF, Schoenborn CA. Health behaviors of adults: United States 2002–2004. Vital Health Stat 2006;10(230). Available at http://www.cdc.gov/nchs/data/series/sr_10/sr10_230.pdf.

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 1. 10 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | ProMED 20061214-3510, 2006 Dec 14. 情報源:[1]Public Health Agency of Canada , 2006 Oct 28. [2]Canadian Cooperative Wildlife Health Centre, 2006 Oct 18. [3]USA CDC, 2006 Dec 11. [4]USA CDC, 2006 Dec 11. | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 米国・カナダ |

**研究報告の概要**

○ウエストナイルウイルス最新情報2006年—西半球(第21回)
[1]カナダ—ヒト症例サーベイランス
11月以降新たな感染例は報告されていない。
[2]カナダ—鳥類サーベイランス
11月以降新たな感染例は報告されていない。
[3] 米国—CDC/Arbonet
2006年、米国におけるウエストナイルウイルス感染のヒト症例は43州から4052例が報告され、うち1396例で脳炎や髄膜炎を発症、死亡例は146例だった。
[4] 米国—USGS/CDC作成の地図
また、動物からのウイルス検出は、ウマ:35州、トリ:43州、蚊:39州から報告されている。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 2006年、米国におけるウエストナイルウイルス感染のヒト症例は42州から4052例が報告され、うち1396例で脳炎や髄膜炎を発症、死亡例は146例だったとの報告である。 | 輸血によるWNV感染リスクを防止するため、国の指示(平成16年7月13日付薬食発第0713008号「ウエストナイルウイルス等の輸入感染症対策に係る採血禁止期間の変更について」)により帰国(入国)後4週間の供血を禁止している。また、WNV感染の発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき、緊急対応の準備を進めている。 |

**Archive Number** 20061214.3510
**Published Date** 14-DEC-2006
**Subject** PRO/AH/EDR> West Nile virus update 2006 - Western Hemisphere (21)

WEST NILE VIRUS UPDATE 2006 - WESTERN HEMISPHERE (21)
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

In this update:
[1] Canada - human surveillance
[2] Canada - bird surveillance
[3] USA - CDC/Arbonet
[4] USA - USGS/CDC maps

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[1] Canada - human surveillance
Date: 28 Oct 2006
From: ProMED-mail <promed@promedmail.org>
Source: West Nile Virus Monitor, Public Health Agency of Canada [edited]
<http://www.phac-aspc.gc.ca/wnv-vwn/mon-hmnsurv_e.html>

[There have been no changes since the previous update (West Nile virus update 2006 - Western Hemisphere (17) Archive No. 20061109.3221). - Mod.TY]

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[2] Canada - bird surveillance
Date: 18 Oct 2006
From: ProMED-mail <promed@promedmail.org>
Source: Canadian Cooperative Wildlife Health Centre [edited]
<http://wildlife1.usask.ca/en/west_nile_virus/current_maps/canada06en.jpg>

[There have been no changes from the previous update (Archive No. 20061109.3221). Apparently, West Nile virus transmission has declined to undetectability for the year 2006 in Canada. - Mod.TY]

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[3] USA - CDC/Arbonet
Date: 11 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: USA CDC, Division of Vector-Borne Infectious Diseases, West Nile Virus [edited]
<http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount06_detailed.htm

Human Cases have been reported from:
State / Neuroinvasion* / *West Nile* fever** / Other*** / Total **** / Fatalities
Alabama / 7 / 0 / 0 / 7 / 0
Arizona / 48 / 58 / 42 / 148 / 6
Arkansas / 23 / 5 / 0 / 28 / 3
California / 79 / 182 / 11 / 272 / 6
Colorado / 63 / 269 / 0 / 332 / 7
Connecticut / 7 / 2 / 0 / 9 / 1
District of Columbia / 0 / 1 / 0 / 1 / 0
Florida / 3 / 0 / 0 / 3 / 0
Georgia / 2 / 5 / 1 / 8 / 1
Idaho / 111 / 752 / 26 / 889 / 14
Illinois / 116 / 70 / 24 2/26 10 / 9

Indiana / 26 / 7 / 42 / 75 / 3
Iowa / 21 / 13 / 2 / 36 / 0
Kansas / 17 / 13 / 0 / 30 / 4
Kentucky / 5 / 1 / 0 / 6 / 1
Louisiana / 89 / 83 / 0 / 172 / 8
Maryland / 7 / 1 / 2 / 10 / 0
Massachusetts / 2 / 1 / 0 / 3 / 0
Michigan / 47 / 2 / 2 / 51 / 6
Minnesota / 30 / 35 / 0 / 65 / 3
Mississippi / 87 / 93 / 0 / 180 / 13
Missouri / 47 / 12 / 1 / 60 / 3
Montana / 12 / 21 / 1 / 34 / 0
Nebraska / 43 / 212 / 0 / 255 / 1
Nevada / 34 / 75 / 14 / 123 / 1
New Jersey / 2 / 2 / 1 / 5 / 0
New Mexico / 3 / 5 / 0 / 8 / 0
New York / 16 / 7 / 0 / 23 / 4
North Dakota / 20 / 117 / 0 / 137 / 1
Ohio / 36 / 11 / 0 / 47 / 4
Oklahoma / 27 / 18 / 2 / 47 / 5
Oregon / 7 / 50 / 12 / 69 / 0
Pennsylvania / 8 / 1 / 0 / 9 / 2
South Carolina / 1 / 0 / 0 / 1 / 0
South Dakota / 38 / 75 / 0 / 113 / 3
Tennessee / 15 / 2 / 0 / 17 / 1
Texas / 214 / 105 / 0 / 319 / 28
Utah / 56 / 101 / 0 / 157 / 5
Virginia / 0 / 0 / 4 / 4 / 0
Washington / 0 / 3 / 0 / 3 / 0
West Virginia / 1 / 0 / 0 / 1 / 0
Wisconsin / 11 / 9 / 0 / 20 / 1
Wyoming / 15 / 40 / 10 / 65 / 2
TOTALS / 1396 / 2459 / 197 / 4052 / 146

* Cases with neurologic manifestations (such as WN encephalitis, meningitis, and myelitis).
** Cases with no evidence of neuroinvasion.
*** Cases for which insufficient clinical information was provided.
**** Total number of human cases of WNV illness reported to ArboNET by state and local health departments.

Neuroinvasive disease refers to severe disease cases, particularly West Nile meningitis and West Nile encephalitis.

West Nile fever refers to typically less severe cases that show no evidence of neuroinvasion.

West Nile fever is not currently on the list of nationally notifiable diseases, and therefore, it is optional whether or not state health departments report these cases to CDC. Click the above CDC site [URL above] for further explanations of neuroinvasive West Nile virus disease and West Nile fever.

Other Clinical includes persons with clinical manifestations other than WN fever, WN encephalitis or WN meningitis, such as acute flaccid paralysis. Unspecified cases are those for which sufficient clinical information was not provided.

Total Human Cases Reported to CDC: These numbers reflect both mild and severe human disease cases occurring since 1 Jan 2006 reported to ArboNet by state and local health departments. ArboNet is the national, electronic surveillance system established by CDC to assist states in tracking West Nile virus and other mosquito-borne viruses. Information regarding 2006 virus/disease activity is posted when such cases are reported to CDC.

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[4] USA - USGS/CDC maps
Date: 11 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: USA CDC, Division of Vector-Borne Infectious Diseases, West Nile Virus [edited]
<http://diseasemaps.usgs.gov/wnv/wnv_us_human.html>

2006 West Nile virus activity in the United States (through 11 Dec 2006)

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Data are being collected from state and local health departments on a weekly basis and are reported to the CDC ArboNET for the following 5 categories: wild birds, sentinel chicken flocks, human cases, veterinary cases, and mosquito surveillance. Maps detailing county-level wild birds, sentinel chicken flocks, human cases, veterinary cases, and mosquito surveillance data are published each week on the collaborative USGS/CDC West Nile virus website.

As of 11 Dec 2006, human, avian, animal or mosquito WNV infections have been reported to CDC ArboNET from the following states: Alabama, Arizona, Arkansas, California, Colorado, Connecticut, Delaware, District of Columbia, Florida, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, Washington, West Virginia, Wisconsin, and Wyoming.

WN virus antibody-positive sentinel animals (birds and/or horses) have been found in Arizona, Arkansas, California, Delaware, Florida, Iowa, Montana, Nevada, North Carolina, North Dakota, Pennsylvania, Utah and Virginia. [Not every state has a sentinel program. - Mod.JW]

WN equine infections have been reported from Alabama, Arizona, Arkansas, California, Colorado, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Jersey, New Mexico, New York, North Carolina, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Tennessee, Texas, Utah, Virginia, Washington, Wisconsin and Wyoming.

WN virus has been detected in dead wild birds in Alabama, Arizona, Arkansas, California, Colorado, Delaware, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, South Carolina, South Dakota, Tennessee, Texas, Utah, Vermont, Virginia, West Virginia, Washington, Wisconsin, and Wyoming.

WN virus has been detected in mosquito pools collected in Arizona, Arkansas, California, Colorado, Connecticut, District of Columbia, Georgia, Idaho, Illinois, Indiana, Iowa, Kansas, Kentucky, Maryland, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, Nevada, New Jersey, New Mexico, New York, North Dakota, Ohio, Oklahoma, Oregon, Pennsylvania, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Utah, Virginia, West Virginia, Wisconsin, and Wyoming.

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ProMED-mail
<promed@promedmail.org>

[ProMED-mail readers should note the change in URL for the USGS/CDC weekly maps. Maps for other arthropodborne viruses in the USA are covered at this site also, including St Louis encephalitis, eastern equine encephalitis, western equine encephalitis, La Crosse encephalitis, and Powassan. The URL for all of these maps is <http://diseasemaps.usgs.gov/>. - Mod.TY]

[see also:
West Nile virus update 2006 - Western Hemisphere (20) 20061207.3452
West Nile virus update 2006 - Western Hemisphere (19) 20061116.3282
West Nile virus update 2006 - Western Hemisphere (18) 20061109.3221
West Nile virus update 2006 - Western Hemisphere (17) 20061101.3129
West Nile virus update 2006 - Western Hemisphere (16) 20061027.3075
West Nile virus update 2006 - Western Hemisphere (15) 20061019.3006
West Nile virus update 2006 - Western Hemisphere (10) 20060915.2606
West Nile virus update 2006 - Western Hemisphere (05) 20060803.2147
West Nile virus update 2006 - Western Hemisphere (01) 20060624.1755
2005
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West Nile virus update 2005 - Western Hemisphere (22) 20051226.3686]

.....ty/msp/sh

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 1. 22 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | Stramer SL, Custer B, Busch MP, Dodd RY. Transfusion. 2006 Dec;46(12):2036-7. | 公表国 米国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

○供血者のウエストナイルウイルス検査方針
ウエストナイルウイルス(WNV)が輸血感染するとの認識により、米国とカナダではウイルスRNAによる供血者の検査が迅速に導入された。最近の分析ではこの検査は費用対効果が低いと指摘されている。Custerらは、ミニプールNATと一部個別NATを組み合わせた通年の検査は、費用対効果は低いが血液安全のためには最善の選択であるとしている。一方Korvesらは、検査の削減を提唱している。検査の効率性を問う必要はあるが、WNVスクリーニングを行う他の方法がないかを検討することも重要である。
供血者のWNV感染は、米国では5月1日から12月1日までRNA陽性の血液が検出される。カナダでは6月1日から11月30日にかけて検査を行い、他の期間は米国への渡航歴によって排除しているが、この方針は、そのまま米国に導入することは出来ない。感染が疑われる症状によって排除する方法は、有効性が低いため規定から除外された。FDAが現行の方針の変更を許可するかどうかは不明である。FDAの関心は安全な血液の供給、つまりリスク対効果に集中しており、費用対効果の面はあまり考慮されない。
また検査を減らすと大幅に費用が削減されるという想定も、検査試薬の単価、検査に要するリソースや人員、コンピュータシステムなどの面から検証する必要がある。
全国で、期間限定の「サーベイランス」としてミニプールNATを行う方法は、他の方法では得られない情報を得ることができるため、公衆衛生の向上にも役立つものである。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 供血者のウエストナイルウイルス検査方針については、費用対効果の面から検討が必要であるとの報告である。 | 輸血によるWNV感染リスクを防止するため、国の指示(平成16年7月13日付薬食発第0713008号「ウエストナイルウイルス等の輸入感染症対策に係る採血禁止期間の変更について」)により帰国(入国)後4週間の供血を禁止している。また、WNV感染の発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき、緊急対応の準備を進めている。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

# EDITORIAL

## Strategies for testing blood donors for West Nile virus

The recognition that West Nile virus (WNV) was transmissible by transfusion led to the rapid implementation of routine donor testing for viral RNA in the United States and Canada. Recent analyses have indicated that this intervention, like others used to enhance blood safety, does not meet currently accepted thresholds for cost-effectiveness. As reported by Kuehn,[1] this raises the question of the use of alternate strategies for WNV screening. In her article, she reviewed the current status of WNV testing in the United States, with particular reference to two cost-effectiveness studies. In the first, Custer and coworkers[2] analyzed a number of testing strategies involving temporal and geographic variations in the use of minipool and single-donation testing including the absence of testing, whereas Korves and associates[3] analyzed strategies varying from the absence of testing, testing only for immunocompromised patients, seasonal testing, and testing methods as used currently.[3] Custer and coworkers concluded that, although not the most cost-effective approach, the current mix of year-round minipool testing and targeted single-donation testing offered the best way of assuring blood safety. Korves and coworkers, on the other hand, favored those strategies that minimized or eliminated testing. Kuehn interviewed both authors and found that Korves had concluded that the financial benefits of preventing transfusion transmitted WNV did not justify the cost, whereas Custer had pointed out that the issue was more appropriately seen as one of the balance of risks and benefits. Although it is certainly of interest and relevant to question the reasons for the acceptability of disproportionately high cost-effectiveness ratios for blood safety, it is more important to ask whether alternate strategies for WNV screening are, in fact, viable. Strategies that appear intuitive may not be appropriate from the perspectives of regulation, cost, or logistics.

WNV infection is seasonal in nature, although the season in the US is longer than many presume, with RNA-positive donations detected as early as May 1st and as late as December 1st. Although seasonal testing for WNV from June 1 to November 30 has been introduced in Canada, it is unlikely to be acceptable in the United States. This is because the WNV-free period is short—indeed, the virus is thought to be a risk to humans year-round in some areas. In addition, blood donors are often mobile and may be exposed in one area, but give blood at another, distant location. During the off-season, donors in Canada are tested if they have traveled to the United States, but a simple geographic exclusion of this type is not practical within the United States. Korves and colleagues suggested that safety could be achieved by relying on identification of suspected WNV infection with a donor questionnaire in times or places without testing, but it is clear that questioning donors about symptoms associated with WNV infection was of little value and therefore has now been eliminated as a regulatory requirement.[4] Although the FDA does not actually require testing for WNV RNA at this time, the agency does have control over the way that testing is performed through their oversight of the IND process currently governing testing and through the licensure and use of approved testing systems. It is not clear that the FDA would permit modifications to the current program of year-round testing without appropriate measures to safeguard against break-through recipient infection. As stated in the perspective of Kuehn, FDA's concern is the safety of the blood supply and as such the focus is risk versus benefit, not cost versus benefit.

It is also important to examine the underlying assumption that reducing testing will materially reduce costs. Reagents themselves are priced according to usage volume and a reduction in usage will not generate a directly equivalent reduction in cost. It is difficult to predict the volume of reagents required for any given year; the trend of WNV infection in donors has not been monotonic. One must also consider the consequences of underas well as overestimating the necessary number of tests. Also, testing requires a substantial overhead in terms of resources and personnel: overhead that is fixed in the short term and cannot be readily turned on or off. Similar arguments can also apply to geographically restricted testing. Because the length of the season and the location of outbreaks are not amenable to an accurate forecast, the capacity for maximal testing must be retained. Trigger strategies have been developed that appear to ensure safety,[5,6] but the actual logistics of starting and stopping testing are complex and could lead to errors because computer systems in place in most blood systems cannot be modified to switch from "marker on" to "marker off." The consequences of inappropriate testing (or more correctly, the absence of appropriate testing) and inappropriate release of an untested unit to a susceptible recipient must be considered. Indeed, this point has been made by AuBuchon:[7] "In blood collection agencies, the tightly controlled computer systems that are optimized to prevent erroneous release of potentially infectious components have difficulty accommodating testing for WNV only during a portion of the year, thus forcing use of the less cost-effective

TRANSFUSION 2006;46:2036-2037.

approach of year round testing." Although the concept of testing only for immunocompromised patients does have a precedent in the case of cytomegalovirus (CMV), there are considerable differences. The underlying prevalence of CMV antibodies in the donor population, which is greater than 50 percent, makes universal testing impossible. Additionally, populations at risk for CMV disease are much better characterized than are those for WNV. Indeed, in the study by Pealer and associates,[8] of the 14 transfused patients investigated as a direct result of WNV disease, only 8 had primary diagnoses that would have been formally associated with immune compromise. Logistically, maintenance of a dual-tiered blood inventory, WNV tested and untested, is yet another source of potential errors. In addition, it is now clear that, within the hospital environment, selective transfusion protocols create operational difficulties and frequently fail to meet their objectives. Patients who should receive specially treated or selected blood components do not necessarily get them.[9] Consequently, Korves and coworkers' suggested strategy neither would identify all patients at risk, nor would it assure that at-risk patients would receive WNV-tested components.

Both Custer and Korves agree that uniform testing at the single-donation level is least cost-effective, but Custer ultimately favors the current approach of testing in minipools with focused individual testing where and when a trigger strategy justifies such action. The nationwide and time-independent "surveillance" system with blood donations in a minipool approach provides an effective and sensitive early warning of the emergence of human WNV infection in a given area—information that may not otherwise be available. In a recent analysis, Stramer and coworkers have shown that, of 1332 potentially infectious blood donations tested during the years 2003 to 2005, 22 percent, or 294 viremic units, were detectable only by single-donation testing.[10] This targeted testing serves as a bridge strategy between no testing and very expensive year-round individual-donation testing throughout the country, and it may serve as a model of testing for new agents if the disease epidemiology is clearly related to specific geographic regions. This approach may not be the least costly but it does achieve blood safety and public health goals.

The effectiveness of the current strategy is underlined by Montgomery and coworkers'[11] analysis of the impact of WNV testing over the period 2003 to 2005. During the first of these years, when testing was restricted in many blood centers to minipools, a total of six cases of apparent transfusion-associated transmission of WNV occurred. In the subsequent 2 years, only one such case was identified. That case occurred in an area with a very high incidence of WNV infection, but before the blood system in that area had completed the implementation of their single-donation "triggering" system. Although the size of the epidemic was smaller in 2004 and 2005 than in 2003, it is nevertheless likely that the strategy of focused single donation testing has been associated with the absence of transmission.

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番号16

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年2月23日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | | 研究報告の公表状況 | YOMIURI ONLINE/20070222 | 公表国<br>日本 | |
| 販売名<br>（企業名） | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | | | |

**研究報告の概要**

千葉県の船橋市立医療センターは２２日、同県内の５０歳代の男性が、主に牛の病気の原因とされる「気腫疽菌（きしゅそきん）」に感染し、死亡したことを明らかにした。気腫疽菌は破傷風菌の仲間で、通常は土の中などに存在する。人への感染が報告されたのは世界初という。
同センターによると、気腫疽菌は、傷口などから動物の体内に入り、筋肉が壊死（えし）する「気腫疽」を発症させる。若い牛や羊に発症例が多く、致死率は非常に高いとされる。死亡した男性は２００６年２月、高熱と胸の打撲傷で、同センターに搬送された。男性は搬送時、既に心肺停止状態で、すぐに死亡。のどの炎症を起こし、体全体が膨れ上がり、特に肺の膨張が著しかったため、男性の肺の組織を調べると、気腫疽菌が検出され、肺の筋肉が壊死していた。同センターは、「気腫疽菌は人には感染しないというのがこれまでの『常識』だった。くわしい感染経路を調べることが今後の課題」としている。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 世界初のヒトへの気腫疽菌感染報告である。<br>気腫疽菌(Clostridium chauvoei)は、0.5～0.7×3～6μmの動物の腸管内生息菌である。万一、原料血漿に気腫疽菌が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**使用上の注意記載状況・その他参考事項等**

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

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# 牛「気腫疽菌」、世界初の人感染…千葉で男性死亡

千葉県の船橋市立医療センターは22日、同県内の50歳代の男性が、主に牛の病気の原因とされる「気腫疽菌（きしゅそきん）」に感染し、死亡したことを明らかにした。

気腫疽菌は破傷風菌の仲間で、通常は土の中などに存在する。人への感染が報告されたのは世界初という。

同センターによると、気腫疽菌は、傷口などから動物の体内に入り、筋肉が壊死する「気腫疽」を発症させる。若い牛や羊に発症例が多く、致死率は非常に高いとされる。

死亡した男性は2006年2月、高熱と胸の打撲傷で、同センターに搬送された。男性は搬送時、既に心肺停止状態で、すぐに死亡。のどの炎症を起こし、体全体が膨れ上がり、特に肺の膨張が著しかったため、男性の肺の組織を調べると、気腫疽菌が検出され、肺の筋肉が壊死（えし）していた。

同センターは、「気腫疽菌は人には感染しないというのがこれまでの『常識』だった。くわしい感染経路を調べることが今後の課題」としている。

（2007年2月22日22時41分 読売新聞）

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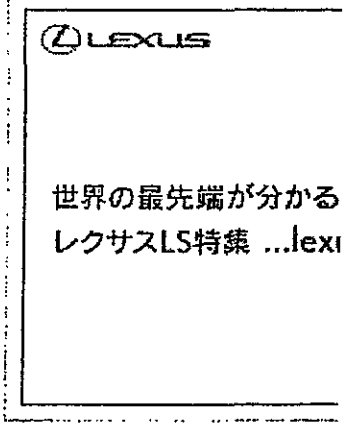


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別紙3

# 研　究　報　告　調　査　報　告　書

| | | | | | |
|---|---|---|---|---|---|
| 識別番号・報告回数 | | | | 第一報入手日<br>：平成19年2月23日 | 新医薬品等の区分<br>：該当なし |
| 一　般　的　名　称 | － | | 研究報告の公表状況 | － | 公表国：<br>日本 |
| 販　売　名　（企　業　名） | － | | | | |

| | |
|---|---|
| 研究報告の概要 | 問題点（ヒトに「気腫疽菌」世界初確認）<br>千葉県船橋市立医療センターは，2006年2月に搬送され，死亡した50代の男性から，気腫疽菌が検出されたと2月22日に発表した．ウシやヒツジなどの家畜において日本を含む世界中で見られ，家畜伝染病予防法に指定されているが，ヒトへの感染が確認されたのは初めて．<br>同センターによると，男性は昨年2月，土木作業中に打撲した肺が大きく腫れて心肺停止状態となり，センターに搬送されてから約2時間後に死亡した．胸の組織内にガスがたまって筋肉が壊死した状態で，同センターと国立感染症研究所が調べたところ，気腫疽菌が検出された． |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本報告は，当該生物由来製品による感染症情報ではない．<br>本報告は，新規感染症と推考されることから安全対策上報告することにした． | 今後も感染症情報の収集に努め，当該生物由来製品に係る情報を入手した場合には速やかに調査・報告を行い安全性の確保に努める． |

総合機構処理欄

使用上の注意記載状況等・その他参考事項等



29

# ヒトに「気腫疽菌」世界初確認　船橋で死亡の男性

02/23 10:33 ■この記事についてのブログ(3) この記事について ブログを書く

千葉県船橋市の市立医療センター(小沢俊院長)は２２日、昨年２月に同センターで死亡した千葉県内の５０代の男性から「気腫疽(きしゅそ)菌」が検出されたと発表した。この菌は牛や羊の体内にガスがたまる病気「気腫疽」を起こすことが知られているが、ヒトへの感染が確認されたのは世界でも初という。

同センターによると、男性は昨年２月、土木作業中に打撲した肺が大きく腫れて心肺停止状態となり、センターに搬送されてから約２時間後に死亡した。胸の組織内にガスがたまって筋肉が壊死(えし)した状態で、同センターと国立感染症研究所が調べたところ、気腫疽菌が検出された。

当初は風邪のような症状だったが、打撲の部位から腫れだしたという。

土壌中などに存在する気腫疽菌は草食動物への感染が知られ、動物の感染死亡率はほぼ１００％。家畜伝染病予防法で届け出伝染病に指定され、現在はワクチンがあるが、国内で年間数例の動物の発症が報告されている。

感染症の専門家は「菌がヒトにも感染することが分かったのは新たな発見だが、これと別にヒトにガス壊疽(えそ)を引き起こす構造の似た菌が存在しており、新たな病気が出現したことにはならない。危険性が高まったわけでもない」と冷静な対応を求めている。

産經新聞

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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**研究報告の概要**

○インドにおけるチクングニヤの再興:高まる脅威
チクングニヤウイルス(CHIKV)の感染がインドで拡大している。2005年12月以降最も被害の大きい5つの州から896,500人以上のチクングニヤ疑い症例が報告されている。北部の州からは1例も報告されていない。2004年末以降、チクングニヤはインド洋の南西部の島々で流行し、その後マダガスカルとインドでも報告された。インドにおけるチクングニヤは、1963年にコルカタで最初に検出された。1973年のインド西部での流行以降はサーベイランスは実施されておらず、インドから消滅したと考えられてきた。最近の研究では発症患者の約50%がRT-PCRでRNA陽性だったが、実際の発生率ははるかに高いと考えられる。病院に行かない患者が多く、受診してもRNA陽性となるのは1日目から4日目のみで臨床検査は難しい。症状は、38.5～40℃の高熱、筋肉痛、頭痛、関節の腫れと激痛、発症5日以降のかゆみを伴う斑点状丘疹で、多くは自己限定的で1～10日持続した。関節痛は症例の約10%で3週間以上持続し、数ヶ月～数年間続くこともある。温暖湿潤な気候と貯水池は媒介蚊の繁殖に適した環境で、貧しい人々はより感染しやすくなっている。インド洋のCHIKV分離株の遺伝子構造はウイルスが急速に変異することを示唆している。疾患は自己限定的であるが、流行地域への渡航者の感染リスクは引き続き存在する。輸入症例が欧州の多くの国から報告されており、フランスでは2006年3月に血液暴露によると考えられる国内感染例が発生している。媒介蚊の一つであるヒトスジシマカは欧州でも見られるため、ウイルス流入と地域内／持続感染のリスクについてさらに調査が必要である。
抗ウイルス剤とワクチンの開発が急務である。さらなる感染拡大を抑えるための対策の強化が求められる。臨床管理を改善する方法、特に早期検出、患者の栄養補給、その他の予防手段によって症状を大きく緩和できる。

| 報告企業の意見 | 今後の対応 |
|---|---|
| チクングニヤウイルスの感染がインドで拡大しており、ヨーロッパへのウイルス流入と地域内感染が危惧されるとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。今後も引き続き、新たなウイルス等による感染症の発生状況等に関する情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

30

# Resurgence of chikungunya virus in India: an emerging threat

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Since December 2005, an outbreak of chikungunya virus (CHIKV) infection has been ongoing in various states of India (Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu, Madhya Pradesh, Gujarat, Orissa and Kerala) with potential spread to neighbouring states [1,2]. Cases were first recognised and reported in December 2005. In July 2006, India's National Vector Borne Disease Control Programme (NVBDCP) reported a reduction in the number of cases in the affected districts while other districts are now becoming affected for the first time. The spread is of unprecedented magnitude and over 896 500 suspected chikungunya cases have been reported since December 2005 from the five worst affected states (Andhra Pradesh, Karnataka, Maharashtra, Tamil Nadu and Madhya Pradesh) [3]. No chikungunya cases have been reported from the northern states.

Recent large-scale outbreaks of fever caused by CHIKV infection in India have confirmed the reemergence of chikungunya in this part of Indian subcontinent. Since the end of 2004, chikungunya has emerged in the islands of the southwestern Indian Ocean (Comoros, Mauritius, Seychelles and Reunion), where several hundred thousand cases have been reported. Chikungunya was later also reported in Madagascar and in India [4,5]. Chikungunya is not new to the Indian subcontinent. Since it was first detected in Calcutta in 1963 [6], there have been reports of CHIKV infection in different parts of India [7,8,9]. Previously, the most recent Indian chikungunya outbreak was reported in 1973 in western India, in Barsi, Sholapur district, Maharashtra state [10]. Subsequently, there has been no active or passive surveillance carried out in India and it was believed that chikungunya had disappeared from the Indian subcontinent [11,12].

A recent study looked at samples taken from over 140 symptomatic patients with clinical picture of chikungunya who were presented to the Nizam's Institute of Medical Sciences hospital in Hyderabad (the capital of Andhra Pradesh) in March and April 2006. About 50% were found positive for the presence of CHIKV specific RNA (through demonstration of the virus-specific 500 bp amplicon) by reverse transcription-polymerase chain reaction (RT-PCR) [V Lakshmi et al, unpublished data]. However, the true incidence is thought to be much higher, because due to the self-limiting nature of the illness a large proportion of patients did not go to hospital, and even for those who did, laboratory diagnosis proved difficult as RT-PCR was positive for the virus in samples collected between the first and fourth day only, indicating the viraemic phase of the infection. Most patients with acute CHIKV infection presented with high fever (ranging from 38.5°- 40°C), muscle pain, headache and swelling and severe pain in the joints with polyarthralgia (pain in several joints) followed by an itching maculopapular rash five days after onset. Symptoms were generally self-limiting and lasted 1–10 days. Almost 10% of cases reported had prolonged joint pain for more than three weeks. However, joint pain may persist for several months or years. Females were more affected than males, a feature probably associated with the daytime and indoor feeding habits of the mosquito vector in India, *Aedes aegyptii*. All age groups were evenly represented.

Warm, humid climates and water reservoirs serve as an excellent breeding ground for the vector of the virus, *Aedes* mosquitoes. With an increase in temperature, susceptibility of mosquitoes to CHIKV increases [13]. High population density, lack of adequate resources for vector control and hygiene added to the vulnerability of poor people to chikungunya infection. The unique molecular features of the recently analysed Indian Ocean isolates of CHIKV [4] suggest that the virus can evolve rapidly. Studies are in progress to confirm genomic structure and virulence of the recent CHIKV from India.

Although the disease is self-limiting, the risk to non-immune travellers from other parts of the world to areas with a chikungunya epidemic, including India, continues to exist and should be included in the differential diagnosis of travellers returning home with fever. The magnitude of this risk cannot be precisely determined at this time. There is a risk of importing the virus to Europe from affected parts of the world, including Africa and South East Asia, where the virus is endemic. Imported cases have been reported from a number of European countries, including an autochthonous case from France in March 2006, probably contaminated through a blood exposure incident [14]. Considering the extent of the current chikungunya outbreak, the risk of introduction and autochthonous/sustained transmission of the virus in Europe needs further investigation, because one vector, the tiger mosquito *A. albopictus*, is also present in Europe and could increase the likelihood of its future autochthonous transmission in these countries. Various recommendations have been suggested by European experts to ensure the measures to prevent the emergence of imported viral diseases are strengthened in

Europe [5,15]. Pregnant women, families with young children, older people, and those with significant comorbidity should be advised to consult their physician before travelling to the Indian subcontinent, and travellers should be informed about the magnitude of the risk of contracting the disease and decide according to their own judgment. There are no specific preventive medications or vaccines for chikungunya fever, but there are steps travellers can take to reduce risk of being bitten by infected mosquitoes [15]. Despite infecting millions of people worldwide, chikungunya infection has been neglected since its discovery. Worldwide, there are a number of other infections with mosquito-transmitted viruses (arboviruses) with similar symptoms which may be confused with chikungunya, such as Sindbis, Ross River and dengue, and these, together with a detailed travel history, should be considered in the differential diagnosis in returning travellers.

Considering high number of cases, and lack of specific antiviral therapy, it is imperative that specific antiviral agents and vaccine be developed. Although the disease is self-limiting, sustained and intensified control measures (such as regular fogging with pesticides, awareness of the disease and vector, detection and elimination of vector breeding sources, proper facilities for health care and community awareness about the prophylactic measures) are required to control the further spread of the disease. The government of India has taken up necessary steps, in accordance with the NVBDCP guidelines on reducing mosquito breeding sources, use of temephos larvicide in recommended doses, the release of larva-eating fish (*Gambusia*) into the wells and the water bodies to control the mosquito menace and deployment of mobile teams (three teams per district in the affected districts, consisting of epidemiologists, public health specialists, microbiologists and entomologists for assessment of the situation and providing technical assistance and guidelines) and mobilisation of health workers and volunteers [16,17]. Finally, measures to improve clinical management, especially early detection, nutritional support to the affected patients, and other preventive measures may largely mitigate the disease. The wider issues of ecology, current agricultural practices, water management systems, and human behaviour patterns will need to be reviewed. This requires a combination of strategies and we need to proceed with a sense of urgency in this matter.

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back to top

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No. 13

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2007. 1. 26 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | 毎日新聞. 2007 Jan 24. | 公表国 |
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| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

○チクングニヤ熱:日本人女性が感染 国内で初確認

1月24日、厚生労働省はスリランカから帰国した30歳代の女性が、チクングニヤ熱に感染していたと発表した。国内で日本人の感染が確認されたのは初めてである。女性は2006年11月中旬、スリランカで発熱し、現地でチクングニヤ熱かデング熱と診断された。女性はすでに症状は回復し、在住するスリランカに戻っている。

厚労省によると、チクングニヤ熱は発熱や関節炎、発疹などが特徴で、死亡率は極めて低い。蚊を介して感染し、人から人への感染はない。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 日本国内でスリランカから帰国した日本人のチクングニヤ感染が初めて確認されたとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。国内でチクングニヤ感染が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
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3

# チクングニヤ熱：日本人女性が感染 国内で初確認

厚生労働省は24日、スリランカから帰国した30歳代の女性が、チクングニヤ熱に感染していたと発表した。国内で日本人の感染が確認されたのは初めて。女性は昨年11月中旬、スリランカで発熱し、現地でチクングニヤ熱かデング熱と診断された。女性はすでに症状は回復し、在住するスリランカに戻っている。

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毎日新聞 2007年1月24日 東京夕刊

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No. 11

# 医薬品 研究報告 調査報告書

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| | | | | 2007. 1. 24 | 該当なし | |

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**研究報告の概要**

○FDAは最初のシャーガス病スクリーニング検査を認可;AABBは実施のためのガイダンスを整備

2006年12月13日、米国食品医薬品局(FDA)は、シャーガス病の病因であり、致死性感染を引き起こすおそれのある住血原虫Trypanosoma cruziを検出する新しい供血者スクリーニング検査試薬を認可したと発表した。認可されたOrtho T. cruzi ELISA Test Systemは、患者血清あるいは血漿中のT. cruzi抗体を検出する検査試薬で、この種の検査法としては初めてFDAから認可を受けた。供血者の他、臓器や細胞、組織提供者のスクリーニングに使用することも予定されている。

米国血液銀行協会(AABB)は、採血施設が検査導入とその期間を決定し、供血者と受血者のフォローアップのためのガイダンスを提供するのに役立つよう、協会公報#06-08を12月14日に発表した。具体的な勧告内容は、出荷停止、遡及調査、自己血輸血で繰り返し検査陽性となった場合の成分製剤出荷の認可、供血延期措置、通知、確認試験、供血者の医学的評価のための供血延期などの事項が盛り込まれている。

FDAによると、原虫感染が多い地域からの移民の増加によって、米国における輸血や臓器移植によるシャーガス病伝播の危険性は高まっている。AABBは協会公報中で、採血施設は検査導入の是非を決定する際に、採血地域内のメキシコや中南米から移住してきた人の割合を考慮すべきであると勧告している。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| FDAがシャーガス病の供血者スクリーニング検査試薬を初めて認可し、AABBが実施に備えガイダンスを発行したとの報告である。 | 日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。 |

**Volume 12, Number 43**
**December 15, 2006**




# FDA Licenses First Chagas' Screening Test; AABB Offers Guidance for Potential Implementation

The Food and Drug Administration (FDA) announced Dec. 13 that it has granted a license for a new test to screen blood donors for the protozoan parasite *Trypanosoma cruzi*, a blood-borne parasite that causes Chagas' disease, a serious and potentially fatal infection. The screening test, Ortho *T. cruzi* ELISA Test System, detects the *T. cruzi* antibody in a sample of the donor's serum or plasma and is the first such test approved by FDA. In addition to screening people who donate blood, this test is intended for use in screening plasma and serum samples from organ, cell and tissue donors. According to the agency, the Ortho-Clinical Diagnostics test identifies infected donors and can help reduce the risk of disease transmission through blood transfusion or organ transplantation.

"The availability of this test offers an important new safety measure to protect recipients of blood, organs and tissues against a potentially very serious, though uncommon infection," said Jay Epstein, MD, director of the Office of Blood Research and Review in FDA's Center for Biologics Evaluation and Research (CBER).

AABB issued Association Bulletin #06-08 on Dec. 14 to help facilities determine whether to implement a licensed test and the time frame for implementation, as well as to provide guidance for donor and recipient follow-up. The specific recommendations address topics such as quarantine, lookback, and approval for release of components from autologous donors that test repeat reactive; donor deferral, notification, and confirmatory testing; as well as referral of donor for medical evaluation. The recommendations included in this bulletin were developed by the Chagas' Working Group, a subgroup of the AABB Transfusion-Transmitted Diseases Committee.

According to FDA, "concerns about the potential for transfusion and organ-transmitted Chagas' disease in the U.S. have heightened concern due to the increase in the number of U.S. residents who previously lived in countries where infection is common." The association bulletin

Inside *Weekly* ...

AABB Urges FDA Panel to Say No to National Drug Codes for HCT/Ps ... 2

AABB Weekly Newsletter Receives Facelift, Becomes Member Benefit ... 4

Early-Bird Registration Now Open for Hematopoietic Growth Factors Conference ... 4

National Blood Donor Month Materials Available ... 5

Draft Perioperative Standards Public Comments Due Next Month ... 5

Public Comment Period for ISBT 128 Cellular Therapy Documents to Take Place in January ... 5

RegionWatch ... 6

News Briefs ... 7

Upcoming Events ... 9



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notes that the *T. cruzi* parasite is only found in the continental Americas, usually Latin America. The presence of the parasite in the U.S. and Canada is increasing, however, due to higher rates of immigration of individuals from endemic areas. As a result, AABB recommends in the association bulletin that facilities consider the percentage of people in their collection area who have immigrated from Mexico and Central and South America in determining whether to implement a licensed test.

Chagas' disease is typically a mild but chronic infection, usually without symptoms. As noted in the association bulletin, the lifetime risk of severe heart or intestinal problems in infected individuals averages about 30 percent and may occur many years after the initial infection. In some cases, however, infection may lead to organ damage, particularly of the heart and esophagus. Infection in immunocompromised individuals, such as those undergoing cancer therapy or receiving an organ transplant, can cause acute and life-threatening symptoms. Natural infections of Chagas' disease are transmitted mainly when the feces of an infected bug are rubbed into a bug bite, other wound, or directly into the eyes or mucous membranes. Other primary forms of transmission include congenital transmission, organ transplantation, blood transfusion and by ingestion of contaminated foods, although this is rare.

To view Association Bulletin #06-08 in its entirety, visit the AABB Web site at www.aabb.org under "Members Area > Association Bulletins."

# AABB Urges FDA Panel to Say No to National Drug Codes for HCT/Ps

An FDA panel listened this week to concerns raised by professional associations, drug manufacturers, drug distributors, pharmacies and others about one portion of a proposed rule that seeks to make major changes to the National Drug Code (NDC) system, described in 21 CFR Part 207. The proposed rule, published Aug. 29, 2006, in the *Federal Register,* also expands the scope of products subject to NDC system requirements, adding licensed human cells, tissues, and cellular and tissue-based products (HCT/Ps) to the list of covered products.

For decades, FDA has required that each drug product listed with the agency have an NDC number — a unique 10-digit, three-segment number — that identifies the drug's labeler, the specific product and the package size. The drug's labeler, identified by the first number segment, is any firm that manufactures or distributes a drug. The second set of numbers, known as the product code, identifies a specific strength, dosage form and formulation for a particular firm. The third number segment, the package code, identifies package sizes and types. Currently, FDA assigns labeler codes, but labelers assign both the product and package codes.

If the proposed rule becomes final as written, however, FDA would assign *all* segments of the NDC. The agency believes that by taking control of assigning each of these numbers, it would be able to trace back every product to its manufacturer. Also, as the agency prepares to implement electronic listing of drugs, it hopes to avoid having duplicate NDC numbers, which could potentially be a problem once all of the numbers reside in one system.

In comments before the panel, critics of the NDC portion of the proposed rule indicated that waiting for FDA to assign NDC codes could create delays in providing consumers with access to new drugs. In addition, the new process, which also requires NDC numbers to be printed on product labels and to have different product codes when manufacturers change or when information changes, could create inefficiencies and add costs to manufacturers' processes. Heidi Horn, associate director of regulatory


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医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2006年12月25日 | 新医薬品等の区分 | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | FDA News/20061213 | 公表国<br>アメリカ | | |
| 販売名<br>（企業名） | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④コンコエイトーHT（ベネシス）<br>⑤クリスマシンーM（ベネシス） | | | | | |

**研究報告の概要**

米国FDAは本日（2006年12月13日）、重篤且つ致死性の寄生虫感染症のシャーガス病を引き起こす血液由来寄生虫を血液ドナーからスクリーニングする新しい試験を承認した。この試験はORTHO T. cruzi ELISAと呼ばれ、trypanosoma cruzi抗体を検出するもので、FDAによって承認された初めての試験である。
FDAによって検討された試験は99%以上の精度を有し、感染していると考えられるヒトの199の血液検体中198を陽性と検出した。70,000を超えるドナー検体について行われた実地試験では、陽性と誤って確認された検体の数は極めて少なく、100,000件の試験当り僅か2-3件であった。
この試験は、全血の供血者のスクリーニングに加えて、臓器、細胞及び組織ドナーからの血漿及び血清をスクリーニングするのに用いられるよう計画されている。この試験は現時点で、この病気の診断用には承認されていない。

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wfの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人アルブミンを精製し、アルブミン濃度5w/v%に調整した製剤であり、ウイルス不活化を目的として、製造工程において60℃、10時間の液状加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| FDAがシャーガス病を引き起こす血液由来寄生虫を血液ドナーからスクリーニングする新しい試験を承認したとの報告である。<br>血漿分画製剤からのトリパノソーマ原虫伝播の事例は報告されていない。また、万一原料血漿にTrypanosoma cruziが混入したとしても、除菌ろ過等の製造工程において十分に除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

# FDA News

**FOR IMMEDIATE RELEASE**
P06-198
December 13, 2006

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## FDA Approves First Test to Screen Blood Donors for Chagas Disease

The U.S. Food and Drug Administration (FDA) today approved a new test to screen blood donors for a blood-borne parasite that causes Chagas disease, a serious and potentially fatal parasitic infection. The test, called the ORTHO T. cruzi ELISA Test System, detects antibodies to the *Trypanosoma cruzi (T. cruzi)* parasite and is the first such test approved by FDA.

"Our blood supply is now extremely safe from diseases once frequently transmitted by blood, such as HIV. However, we are constantly faced with new threats," said Jesse L. Goodman, MD, MPH, Director of CBER. "Evaluating this test involved a high degree of interaction between FDA scientists, the blood industry and test developers. It is part of an ongoing effort to, wherever possible, identify emerging threats and provide the tools needed to help keep blood safe."

It is estimated that as many as 11 million people are currently infected by T. cruzi, most commonly in parts of Mexico, Central and South America. Most have no symptoms or signs of the disease. The infection is usually acquired from the bite of an infected insect but also can be transmitted through blood transfusions or organ transplants. Early infection is usually mild and unrecognized, but persists lifelong and may lead to organ damage, particularly of the heart and esophagus, causing an estimated 50,000 deaths annually worldwide. Infection also can be severe in people whose immune systems are suppressed, such as organ transplant recipients. Concerns about the potential for transfusion and organ transmitted Chagas disease in the United States have heightened due to the increase in the number of U.S. residents who previously lived in countries where the infection is common. This new test identifies infected donors and therefore can reduce the risk of disease transmission through blood transfusion or organ transplantation.

"The availability of this test offers an important new safety measure to protect recipients of blood, organs and tissues against a potentially very serious, though uncommon infection," said Jay Epstein, MD, Director of the Office of Blood Research and Review in FDA's Center for Biologics Evaluation and Research (CBER).

In studies reviewed by FDA, the test was found to be accurate 99% or more of the time—detecting 198 out of 199 blood specimens from individuals believed to be infected. In field trials of over 70,000 donor samples, the number of individuals falsely identified as positive was extremely small, only 2-3 per 100,000 test results.

In addition to screening people who donate whole blood, this test is intended for use in screening plasma and serum samples from organ, cell and tissue donors. At this time, the test is not approved to diagnose the disease.

The ORTHO T. cruzi ELISA Test System is manufactured by Ortho-Clinical Diagnostics, Inc., Raritan, NJ

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# 医薬品 研究報告 調査報告書

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| 一般的名称 | 新鮮凍結人血漿 | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 新鮮凍結血漿「日赤」(日本赤十字社)<br>新鮮凍結血漿-LR「日赤」(日本赤十字社) | | Young C, Losikoff P, Chawla A, Glasser L, Forman E. Transfusion. 2007 Mar;47(3):540-4. | 米国 |

**研究報告の概要**

○輸血によるTrypanosoma cruzi感染
背景:神経芽細胞腫(ステージ4)を発症した3歳半の女児が複数の血液成分製剤投与を受けた後、Trypanosoma cruziによるシャーガス病と診断された。
試験デザインおよび方法:輸血された製剤の全供血者に対し、再度採血機関に足を運びT. cruzi抗体検査のための血液検体を提供するよう依頼した。
結果:初回供血者1名がT. cruzi抗体陽性であることが判明した。当該供血者は、ボリビア出身であり、17年前に米国に移住した。移住後は母国に帰国していない。
結論:本症例は、米国・カナダでの輸血によるシャーガス病感染の7例目の報告である。ニューイングランド地方では発生が予想されていなかったが、実際には発生した。本症例は、疾患が輸血感染に関係することから患者の免疫状態が重要であること、疾患伝播における地理的移動の影響、米国の血液供給において認可されたシャーガス病スクリーニング検査が必要であることを示している。

**使用上の注意記載状況・その他参考事項等**

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 神経芽細胞腫の3歳半の女児が、17年前にボリビアから米国に移住した供血者由来の血液の輸血を受けた後、Trypanosoma cruziによるシャーガス病と診断されたとの報告である。 | 日本赤十字社は、輸血感染症対策として献血時に海外渡航歴・居住歴の有無を確認し、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。 |

# Transfusion-acquired *Trypanosoma cruzi* infection

*Carolyn Young, Phyllis Losikoff, Anjulika Chawla, Lewis Glasser, and Edwin Forman*

**BACKGROUND:** A 3 1/2-year-old girl with Stage 4 neuroblastoma received multiple blood components and was subsequently diagnosed with Chagas disease, which is caused by *Trypanosoma cruzi*.

**STUDY DESIGN AND METHODS:** All blood donors of the units that were transfused were requested to return to the collection facility for a blood sample to be tested for antibodies to *T. cruzi*.

**RESULTS:** One first-time donor was found to be positive for the presence of *T. cruzi* antibodies. This donor was originally from Bolivia and immigrated to the United States 17 years previously. She had not returned to her native country since her emigration.

**CONCLUSIONS:** This is the seventh reported case of Chagas disease transmission by blood transfusion in the United States and Canada. Although this would not be expected to occur in New England, it did, and this case demonstrates the significance of the immune status of patients as it relates to transfusion-acquired infections, the impact of geographic mobility in disease transmission, and the need for a licensed screening test for Chagas disease for the US blood supply.

## CASE REPORT

A 3 1/2-year-old girl with Stage 4 neuroblastoma presented to a teaching hospital with a temperature of 40°C and neutropenia with a white blood cell (WBC) count of 200 per µL and an absolute neutrophil count of 120 per µL. Her pulse was 124 bpm and her blood pressure was 75/48 mmHg. She had an unusual 2.5- to 3-cm erythematous macule on her left hip, vulvar swelling, and evidence of a marrow biopsy performed on her left iliac crest 1 month before admission. She had no respiratory distress, headache, mental status changes, vomiting, or diarrhea. She complained of mild abdominal pain consistent with her operative history. Before her admission, she had completed five courses of nonmyeloablative chemotherapy that had required platelet (PLT) support, and she had gross disease seen on exploratory laparotomy performed 2 weeks previously. One month before her admission she had undergone a peripheral blood progenitor cell (PBPC) harvest. She never received these PBPCs.

A blood smear was performed due to the hospital policy of performing a complete blood count with a manual differential when a patient is neutropenic. The technologist examining the slide noted some unusual parasites. The clinical pathologist, LG, reviewed the slide and identified the parasites as trypomastigotes of *Trypanosoma cruzi*, the organism that causes Chagas disease (Fig. 1). The hospital blood bank physician reported transfusion-associated Chagas disease to the blood collection physician and gave the unit numbers transfused.

The patient was given 15 mg per kg per day nifurtimox divided in three daily doses after *T. cruzi* was identified on the blood smear. The child's parasite load dropped from 500,000 to 800 per µL over the first 48 hours of therapy, determined by a manual count with a hemocytometer counting chamber, and became undetectable after 14 days of treatment. *T. cruzi* antibody titer was positive 3 weeks after discharge at 1:64 and 3 months later increased to 1:1024. Although the diagnosis was made by identification of the parasite in the peripheral blood, further testing by polymerase chain reaction (PCR), immunofluorescent antibody assay antibody testing, and culture were performed for the purpose of following the patient's response to treatment (Table 1). After discharge

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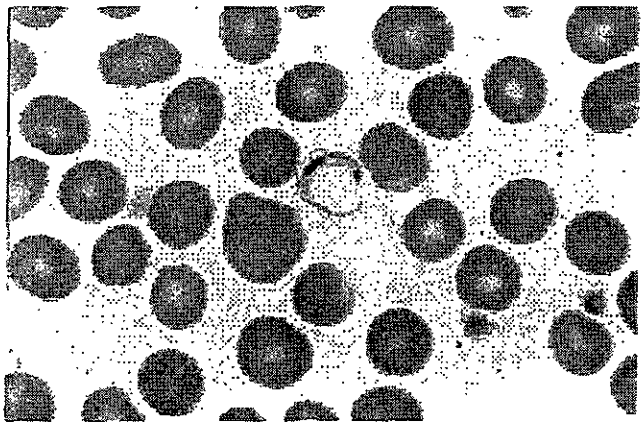

**Fig. 1. Peripheral blood smear showing a typical trypanosome. The organisms usually appear C- or U-shaped with a centrally placed nucleus and a dotlike kinetoplast at the posterior end (Wright's stain; original magnification, ×500).**

from the hospital she continued to receive chemotherapy with topetecan and cyclophosphamide in 4- to 6-week cycles. Nifurtimox was continued beyond the standard 3 months' course due to her compromised immune status. An autologous PBPC transplant was not performed based on her gross disease burden and concern about further immunocompromise. Three years after the diagnosis of Chagas disease, the patient expired in December 2005 due to progression of her primary disease.

## MATERIALS AND METHODS

Seventy-eight units of leukoreduced and irradiated PLT concentrates and red cells (RBCs) had been transfused to this patient. All 78 donors were asked to return to the blood center for samples to be collected for testing for antibodies to *T. cruzi* performed by the Centers for Disease Control (CDC). Individual donor registration records were reviewed. We found that one donor completed a Spanish registration form and one completed a Portuguese registration form. The donor who had completed the Spanish registration form did not respond to written requests in both English and Spanish to return for testing. Phone calls to the donor's home revealed that the phone had been disconnected with no forwarding information. Spanish-speaking staff were sent to her home to obtain samples for testing because the medical director thought that she was the most likely source of transmission after reviewing all the donor registration records and she had not returned to the blood center. The CDC performed an immunofluorescent antibody assay for *T. cruzi*, which was positive at 1:256 on a sample that was obtained about 12 weeks after donation. Consignees of the RBCs and fresh-frozen plasma (FFP) were contacted. The FFP had been discarded at the hospital approximately 1 week after its receipt due to issues unrelated to this case. The recipient of the RBC unit was immunocompetent and was contacted by the hospital. A blood smear was performed that did not reveal any parasites. Antibody testing was planned; however, the patient did not follow through. He had a myocardial infarction in 2003. This recipient was last seen at the hospital in June 2006.

Additional samples were acquired from the donor for PCR testing on one subsequent occasion, approximately 14 weeks after donation, which was negative. PCR was performed with primer pairs that amplify the minicircle sequence, the flagellar protein locus, and the nuclear repeat sequence.[1,2] These additional samples were acquired from the donor at her home with a blood center staff member who had emigrated to the United States from Bolivia and the blood center medical director, CY. The donor's history was taken at the time these samples were obtained, with his translation assistance.

Of the 78 donors, 48 returned for testing. Forty-seven were negative, one was seropositive for *T. cruzi* as stated, and 30 did not return for testing. Telephone screening for travel history and vertical transmission ruled out any risk for *T. cruzi* in 20 of these donors. The remaining 10 donors did not respond to letters and were not reachable by phone. All transfused units had been leukoreduced before storage and irradiated with 2500 cGy.

### Implicated donor

The implicated donor was a 50-year-old, first-time donor in the United States who came from Bolivia 17 years earlier. She had donated once before over 20 years prior, in Bolivia, as a directed donor for the child of a friend. She had never returned to Bolivia or other endemic Chagas disease areas. She had never been diagnosed with Chagas disease. When shown the picture of the reduviid bug, she immediately recognized it and said she had seen many in Bolivia. She recalled that she had been screened for Chagas disease a few times in her childhood by visiting health-care teams in her village and that the results had been negative. Her travel history included trips to Paraguay in her youth. Her medical history was not significant for any symptoms of Chagas disease, except occasional constipation, which she had attributed to her age and diet. After the CDC results were obtained, the donor was followed by her physician in consultation with the CDC. Her PLT concentrate was transfused to the patient approximately 6 weeks (September 4, 2002) before the child presented with symptoms of fever and neutropenia (October 19, 2002) and was diagnosed with Chagas disease.

## DISCUSSION

In endemic areas, that is, rural areas of Mexico and Central and South America, *T. cruzi* is transmitted when mucous

**TABLE 1. Patient's results for *T. cruzi***

| Date collected* | Peripheral blood | Titer† | Culture | PCR |
|---|---|---|---|---|
| Presentation | Positive | Negative | Positive | Positive |
| 2 weeks | Negative | 1:32 | Not done | Weak positive |
| 4 weeks | Negative | 1:64 | Negative | Positive |
| 6 weeks | Negative | 1:64 | Negative | Weak positive |
| 10 weeks | Negative | 1:256 | Positive | Positive |
| 14 weeks | Negative | 1:1024 | Positive | Positive |
| 16 weeks | Negative | 1:1024 | Negative | Negative |
| 20 weeks | Negative | Not done | Not done | Negative |
| 23 weeks | Not done | Not done | Negative | Negative |
| 6 months | Not done | 1:512 | Negative | Negative |
| 3 years, 2 months | Negative | 1:32 | Not done | Negative |

* Date of specimen collection recorded with respect to initial presentation.
† Antibody titers measured by indirect immunofluorescence performed by the CDC; a positive result is considered equal to or greater than 1:64.

membranes are contaminated by feces of infected triatomine bugs. Congenital infection by vertical transmission, transmission by blood transfusion, and organ transplantation are well described modes of infection in endemic areas. The acute illness, usually a childhood illness, is mild with nonspecific symptoms; however, meningoencephalitis and myocarditis can occur in the acute stage of infection. Infected persons then enter an intermediate phase, which is generally asymptomatic and characterized by elevated *T. cruzi* titers and intermittent parasitemia. Decades later 30 percent of those chronically infected will develop cardiomyopathy or gastrointestinal manifestations such as megaesophagus or megacolon. Patients with chronic *T. cruzi* infection who become immunosuppressed may have a reactivation of acute disease which can be more severe than in immunocompetent individuals.[3]

Despite a handful of cases of Chagas disease acquired in the United States, the United States is not considered endemic for *T. cruzi*.[4] Millions of immigrants from *T. cruzi*-endemic areas, however, live in the United States, and it is estimated that 50,000 to 100,000 of these people are chronically infected with *T. cruzi*.[5] These chronically infected individuals are a potential source of *T. cruzi* transmission via blood transfusion or organ transplant.[6] Because estimates for seroprevalence of *T. cruzi* antibodies in selected US blood donor populations varies widely and ranges from 0 to 4.9 percent, the risk for receiving a *T. cruzi*-contaminated unit may be different geographically.[7-11] A conservative estimate of the nationwide risk for transfusion acquired Chagas disease suggests that more than 600 potentially infectious components are entering the US blood supply annually (D. Leiby, AABB 2004 meeting). This translates into many more possible *T. cruzi* infections that are going undiagnosed in transfusion recipients, even without 100 percent transmission.

The US Census Bureau reported that the US resident population was 281,421,906 in 2000. Of this population, Hispanics accounted for 12.5 percent, up from 9 percent in the 1990 census, making it the fastest growing minority group in America. In response to the growing Hispanic population in Rhode Island, the blood center initiated blood donor recruitment targeting this group a few months preceding this case. Recruitment efforts included translating the donor registration record and predonation brochure into Spanish, hiring Spanish-speaking personnel, and adding Spanish to our automated phone answering tree. As a result of these efforts, our Hispanic donor base increased. This individual never gave blood in the United States before this donation and gave blood at a blood drive targeted toward Hispanic donors. Although New England would not be considered a high-risk area, compared to Los Angeles, Texas, or possibly New York, our donor, who resided in Rhode Island, was seropositive for the presence of Chagas disease. Nationwide, the number of Hispanic donors will likely increase as recruitment efforts continue to focus on this growing population. In fact, America's Blood Centers, an organization that consists of more than 70 community blood centers, supports a national recruitment program aimed at educating the Hispanic community about donating blood.

Currently, based on donor surveys performed in six regional blood centers, 2.5 percent of the donor population were born in Chagas-endemic areas. This survey did not ask whether the donor's mother was born in Chagas-endemic areas so does not include the risk for congenital infection (D. Leiby, 2004 AABB Meeting).

It has been demonstrated that the majority of blood donors seropositive for the presence of *T. cruzi* antibody have low-level parasitemia (L. Kirchhoff, University of Iowa, Iowa City, IA, personal communication). In this case, the donor did not have detectable parasitemia when tested by PCR by the CDC on a subsequent blood sample obtained approximately 13 weeks after donation. Additional blood samples at various intervals may have detected parasitemia had they been collected. The patient had no risk factor for Chagas disease, however, other than blood transfusions, and further samples were not obtained. We concluded that the donor's parasitemia must have been intermittent or the parasite load below the level of detection by PCR at the time the subsequent sample was obtained, because no risk factor for Chagas disease other than this transfusion was ever determined in the patient.

There is currently no licensed screening test for Chagas disease for blood donations in the United States. Current screening methods for the disease rely on the donor registration interview which asks whether a prospective donor has ever had Chagas disease. Unlike travel

or residence in malarial-endemic areas, which may impose a set deferral interval of 1 to 3 years, a previous diagnosis of Chagas disease incurs an indefinite, that is, permanent, deferral period. Currently, the only registration questions regarding previous residence or travel that impose an indefinite deferral are related to new variant Creutzfeldt-Jakob disease and HIV Group O. Blood screening manufacturers are working on implementing a screening test for Chagas disease based on an antibody assay, and the confirmatory test will likely be another antibody test like radioimmune precipitation assay, rather than a PCR-based test, which may not have the sensitivity required (L. Kirchhoff, Unviersity of Iowa, Iowa City, IA, personal communication). This may delay its launch, because it should be licensed simultaneously to prevent the unnecessary deferral of healthy blood donors who may have a false-reactive screening test.

There have been six previously reported cases of transfusion-acquired Chagas disease in the United States and Canada, all of whom, including our patient, were immunocompromised.[12-16] The majority of these patients suffered a fulminant course of acute Chagas disease. Only one other reported case in addition to ours had an essentially asymptomatic course.[16]

At this time, the recipient of the RBCs from the same blood donation may be in the indeterminate phase of Chagas disease. In the vast majority of patients who have Chagas disease with acute symptoms, the illness resolves in 4 to 8 weeks without treatment, and then they enter the indeterminate phase of infection that is characterized by a lack of symptoms, undetectable parasitemias, but usually detectable antibodies.[17] In this patient, no acute symptoms were seen so detection of parasites by a blood smear would not be expected. Unfortunately, samples for further testing were recommended but not obtained due to the patient not appearing for appointments.

In a recent Chagas disease study in Guadalajara, nine recipients of blood from radioimmune precipitation assay–positive donors provided blood samples for follow-up testing, and four of the nine were radioimmune precipitation assay–positive. Of the four, two had received whole blood, and two had received PLT transfusions.[18] If this recipient of the RBCs has indeterminate Chagas disease, it will be the first incidence of RBCs-associated Chagas disease in North America. Renewed efforts to obtain a sample from this recipient for further study are under way. Although there is a lack of consensus in whether to treat indeterminate and chronic phase, asymptomatic Chagas disease patients, in this patient, it would be helpful given his history of cardiovascular disease, to know whether he has Chagas disease at minimum for the purpose of following his cardiac status.

Had our pediatric patient not been neutropenic, a buffy-coat smear would not have been performed and *T. cruzi* may have been unrecognized. She never exhibited symptoms of Chagas disease. By the same token, other cases of transfusion-acquired *T. cruzi* likely occur in immunocompetent hosts and are not diagnosed due to the mildness and even asymptomatic nature of some acute Chagas disease.

In five of the six previously reported cases of transfusion-acquired *T. cruzi*, the donor was from a country where *T. cruzi* is endemic. This is also the pattern in our case. All of the donors were asymptomatic at the time of blood donation and none of the donors knew they had Chagas disease. In five of the six previous cases, a PLT transfusion was implicated which was also the source of infection in our patient.[12-16] PLT concentrates are prepared under centrifugation conditions that concentrate *T. cruzi* and are also stored under conditions that favor parasite survival (20-24°C; shelf life of 5 days, in enriched plasma). Also, although the component in our case was irradiated with 2500 cGy and leukoreduced (WBC count, $<8.3 \times 10^5$) before storage, transmission still occurred. Therefore, standard-dose irradiation of blood components and prestorage leukoreduction do not eliminate *T. cruzi*.

In one of the six previous cases, neither a specific donor nor a specific unit of blood or blood component was ever found to be the source of the Chagas disease infection.[13] In that case, an immunosuppressed 59-year-old woman with metastatic cancer developed acute and fatal Chagas disease. She had received numerous transfusions but an infected donor was never identified. In that case, her infection was assumed to be acquired from transfusion of a contaminated blood product. Our case would have followed a similar course of assuming that the patient acquired Chagas disease from a blood transfusion without identifying a donor, if staff had not been sent to obtain samples from the implicated donor's home. Immunocompromised patients may receive many transfusions, and the investigation of these cases is dependent on the willingness of donors to return for testing or the ability and resources of blood centers to go to the donors' homes or workplaces to obtain follow-up samples.

In conclusion, our patient is the youngest reported patient in a case of transfusion-acquired *T. cruzi* infection to date in the United States. Although this is only the seventh reported case of transfusion-acquired Chagas disease in the United States and Canada, the low numbers of reported cases may be due to the immunocompetent status of the majority of transfusion recipients. There may be many unreported cases of Chagas disease in immunocompetent individuals who are asymptomatic. As mentioned previously, the incidence of seropositive donors may vary region to region, but the number of potentially infectious components annually reaching the blood supply nationwide may be in the thousands. This case lends support to the view that Chagas disease must be recognized as a threat to the US blood supply. Neither leukoreduction nor irradiation at 2500 cGy prevent trans-

mission of Chagas disease by blood transfusion. A licensed assay is needed to aid in preventing the potential spread of *T. cruzi* to both immunocompromised as well as immunocompetent transfusion recipients.

*Subsequent to this manuscript submission, the FDA licensed an antibody test for *T. cruzi* on December 13, 2006. Confirmatory testing has not been licensed.

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# 医薬品 研究報告 調査報告書

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**研究報告の概要** 265

○フランス国内感染三日熱マラリアの1症例、コルシカ、2006年8月
2006年8月にコルシカ島で診断された三日熱マラリアの1症例である。患者Yはフランス南東部出身の59歳の男性で、2006年の初夏～盛夏にかけて南コルシカのポルトに滞在していた。8月初めに発熱と消化器症状を発症、数日後に入院した。血小板減少症(血小板数40,000/㎣)からマラリアが疑われ、急性三日熱マラリアの治療を受けて回復した。患者はマラリア流行地域への渡航歴はなく、少なくとも10年間空港内に入ったことはなかった。本土からコルシカへは船で移動し、ポルト地区周辺を旅行した。ポルト地区で最近報告されたマラリア症例があるかを調査した。患者Xはマダガスカルの南東部と南西部に滞在後6月末にフランスに到着した。その後コルシカに渡り、ポルトに2週間、7月初めまで滞在した。コルシカに渡って1週間後に発熱、さらに1週間後に入院し、三日熱マラリアと診断された。治療を受けて完治し、ポルトに戻って8月1日まで滞在した。
どちらの症例も、フランス国立マラリア調査センターでP. vivax感染が確認された。後方視的疫学調査では、6月1日～9月4日の間に上記の2例の他は輸入症例のみ4例が検出され、原虫の循環を示唆するような三日熱マラリア症例は発見されなかった。マダガスカルは熱帯熱マラリアと三日熱マラリアの流行地域である。この2症例は、コルシカのハマダラカによってP. vivaxの国内伝播が起こったことを示唆している。ポルト地区で7月27日に蚊の繁殖場所が発見され、この仮説が裏付けられた。
本症例は、この地域で報告されたマラリアの地域内伝播の1972年以来初の症例であり、蚊の根絶と媒介動物対策を最大限の注意を払って行うべきであることを示している。コルシカの住民および旅行者にマラリア予防策を指示する根拠とはならない。系統だった旅行医学的アドバイスが非常に重要である。

| 報告企業の意見 | 今後の対応 |
|---|---|
| フランス、コルシカ島でマラリア流行地域への渡航歴のない患者が三日熱マラリアを発症し、マダガスカル渡航歴のある同じ地区の患者からの地域内伝播が疑われたとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の供血を一定期間延期している(1～3年の延期を行うとともに、帰国後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

35

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A case of *Plasmodium vivax* malaria was diagnosed in Corsica in August 2006. This is the first case of autochthonous transmission of malaria to be reported in the region since 1972 [1]. Malaria is a notifiable disease in mainland France (including the Mediterranean island of Corsica). Rare cases of malaria have been observed after bites from infected mosquitoes that had been imported from endemic areas into airports and sea ports or by transmission via contaminated blood transfusion or tissue grafts [2, 3, 4]. Corsica was endemic for malaria before 1953 and from 1965 to 1971. Because the *Anopheles* mosquitoes in Corsica may still have vectorial competencies for *P. vivax* [6], antivectorial measures (eradication of mosquitoes) are still taken. A small number of imported cases from endemic zones are reported in Corsica each year [1].

Epidemiological and entomological investigations were carried out into the case in August 2006. The patient, Patient Y, was a 59 year old man from southeast France who had stayed in Porto, a département of South Corsica, from early to mid summer 2006. At the beginning of August, he developed a fever and gastrointestinal symptoms, and after several days of illness, he was admitted to hospital. Malaria was suspected because of thrombocytopaenia (40 000 platelets/mm3). The diagnosis was made three days after admission and the patient was treated for acute *P. vivax* malaria and recovered. The patient had never travelled in endemic malaria areas and had not been inside any airport for at least 10 years. He had travelled to Corsica by ship from mainland France and had then travelled overland to the Porto area.

**Possible link to earlier case of imported malaria?**
Further epidemiological investigations found that another recent case of *P. vivax* infection had been reported in the Porto area. Patient X, who had previously visited southeast and southwest Madagascar, arrived in France at the end of June. He then travelled to Corsica, and stayed in Porto for two weeks at the beginning of July. After a week in Corsica, he developed a fever and was admitted to hospital a week later, where the diagnosis of *P. vivax* malaria was made. He was treated and recovered fully, then returned to Porto where he stayed until 1 August. Both cases of *P. vivax* infection were confirmed by the French national reference centre for malaria.

A retrospective epidemiological investigation undertaken from 1 June to 4 September 2006 by medical laboratories in Corsica, did not find any other *P. vivax* malaria cases that would suggest further circulation of the parasite in Corsica. So far, excluding the two *P. vivax* cases in Porto, only four imported malaria cases (one *P. ovale* and three *P. falciparum*) were detected in Corsica during the summer of 2006. This is within the expected range based on available data from previous years. From 4 to 20 September 2006, prospective active surveillance found no other autochthonous malaria cases.

**Discussion**
Madagascar is known to be an endemic country for *P. falciparum* and *P. vivax* [7]. The details of the imported case from Madagascar with onset in July, and of the case with onset in August, caused by the same species (*P. vivax*) and in the same place (Porto), in a patient with no travel to endemic areas suggests autochthonous transmission by local *Anopheles* mosquitoes [8,9].

This hypothesis was supported by the results of the initial entomological investigations in the Porto area, which found a mosquito breeding ground albeit with low numbers of *Anopheles* mosquitoes, in a fountain on 27 July 2006. The breeding ground was treated the same day using larvicide to eradicate the mosquito colony. An entomological investigation around the home of the Patient Y in southeast France at the beginning of September, did not find any *Anopheles* mosquitoes, but could not completely rule out the presence of this mosquito in May and June.

**Conclusion**
This autochthonous *P. vivax* malaria transmission on Corsica in August 2006, probably via the bite of a local *Anopheles* mosquito infected with *P. vivax* from a patient who had acquired infection in Madagascar, appears to have been unique because studies did not find other autochthonous malaria cases between 1 June and 20 September 2006.

This first case of autochthonous malaria in Corsica in 35 years demonstrates the importance of epidemiological surveillance on the island. Similar events occurred in Italy in 1997 with one case of autochthonous *P. vivax* malaria linked to an imported case from India [10] and in Spain in 2001 with one autochthonous *P. ovale* case [11]. This case demonstrates that mosquito eradication and anti-vectorial measures should be carried out with great care. Although autochthonous transmission could occur again, the detection of one exceptional malaria infection transmitted by mosquitoes in Corsica in summer 2006 does not justify proposing individual malaria protection for Corsica's residents and tourists. Systematic travel health advice is very important for individuals who are travelling to tropical countries in order to reduce the risk of people returning with malaria which can be spread by local mosquitoes [1].

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*Centre hospitalier d'Ajaccio, Corse du Sud; D.Bertei*
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back to top

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**研究報告の概要**

○最近のインド・ゴア州からEUへの熱帯熱マラリア輸入症例、2006年12月～2007年1月

インド特に西海岸沿いのゴア州への渡航者において、マラリア患者の増加が報告されている。過去2年間、ヨーロッパからゴア州への渡航者ではマラリア患者は報告されていなかったが、2006年11月後半からゴア州を含むインド地域からのマラリア症例報告数が増加している。2007年1月10日までに、ドイツ、デンマーク、スウェーデンから患者8例が報告された。このうち4例は、ゴア州に2～3週間滞在し、インド国内の他の地域は訪れていなかった。

人気のある冬季リゾート地であるゴア州は、渡航者に関してマラリア感染のリスクが非常に小さいと考えられていた。2006年10月以来のゴアおよびKonokan地域での例年を上回る豪雨が、今雨期中の媒介蚊数の増加と感染伝播の増悪の原因である可能性がある。当面はマラリア伝播のリスクは高まる恐れがあり、この地域への渡航を計画している旅行者は蚊の予防対策を講じるよう勧告される。渡航中あるいは帰国後間もなく具合が悪くなったならば、迅速に医療機関を受診すべきである。

デンマークでは、2006年12月に新たにマラリア予防勧告を発表した。スウェーデンでは旅行者向け勧告の変更はなかったが、スウェーデン感染症研究所は全ての感染症対応当局に書簡を送付し「ゴア州を訪れる予定の旅行者はマラリア予防服薬が考慮されうる。」と表明している。ドイツでは、渡航者に対する勧告の変更はなされていない。また、英国旅行者へのマラリア予防諮問委員会(ACMP)は、予防勧告の変更を発表した。ゴア州、特にPanaji北部地区を訪れると計画していて、渡航中医療機関にアクセスできない可能性がある旅行者に、マラリアリスクの強調、蚊の予防対策の教育、マラリア化学予防を行うべきであると勧告している。

| 報告企業の意見 | 今後の対応 |
|---|---|
| インド、ゴア州への渡航者において、熱帯熱マラリア患者の増加が報告されているとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の供血を一定期間延期している(1～3年の延期を行うとともに、帰国後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。 |

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36

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1. Recent cases of falciparum malaria imported to Europe from Goa, India, December 2006-January 2007
2. Outbreak of legionnaires' disease among tourists staying at a hotel in Phuket, Thailand
3. First isolation of *Clostridium difficile* 027 in Japan
4. A comparison of public domain software for outbreak investigations: Epi Info and EpiData
5. The BURDEN project - Assessing the burden of resistance and disease in Europe

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## Recent cases of falciparum malaria imported to Europe from Goa, India, December 2006-January 2007

T Jelinek[1] (jelinek@bctropen.de), R Behrens[2], Z Bisoffi[3], A Bjorkmann[4], J Gascon[5], U Hellgren[6], E Petersen[7], T Zoller8 on behalf of TropNetEurop, the European Network on Imported Infectious Disease Surveillance R H Andersen[9], Anders Blaxhult[10],

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10 Swedish Institute for Infectious Disease Control, Stockholm, Sweden

An increase in numbers of malaria cases has recently been reported in travellers returning from India, in particular from the state of Goa, on the west coast. These cases have been reported to the European Network on Imported Infectious Disease Surveillance (TropNetEurop, http://www.tropnet.net). In the past two years, there have been no reports of malaria in European travellers to Goa. However, since late November 2006, the malaria surveillance map of the network has shownan unusually intensive signal from India [1], indicating an increase in the number of malaria reports from that region, including Goa.
By 10 January, eight patients had been reported: two in Germany, four in Denmark, and two in Sweden. With the exception of two Danish cases, all patients travelled independently of one another. The two German patients, one of the Swedish patients and one of the Danish patients stayed in Goa for 2-3 weeks and had not visited other regions within India.

**Germany cases 1 and 2**
The two German patients developed severe malaria and were treated in an intensive care unit with

artesunate/doxycycline. Both travellers (a woman in her 50s and a man in his 40s), travelling independently, spent 14 days at two popular coastal resorts in Goa. Neither took any malaria chemoprophylaxis. One traveller developed a hyperparasitaemia of 25%, both had signs of cerebral malaria and acute renal failure. They were admitted to the hospital on December 10 and 11 respectively, and made a full recovery.

**Sweden case 1**
A Swede in his 40s travelled to Goa for two and a half weeks in November-December 2006. He visited a beach in northern Goa and stayed in a beach hotel. He slept under a mosquito net but without air conditioning. He did not take any malaria chemoprophylaxis, in accordance with the Swedish recommendations for travellers to that area [2]. Five days after his return to Sweden, he developed a sudden fever, was diagnosed with a probable pneumonia and treated with amoxicillin/clavulanic acid for three days. With no improvement in his condition he was admitted to hospital seven days later, where a diagnosis of *Plasmodium falciparum* monoinfection with 0.4% parasitaemia was made. This diagnosis was delayed because travel history had not been obtained earlier. The patient was treated with mefloquine and made an uneventful recovery.

**Sweden case 2**
A Swede in her 50s visited Goa for a two week holiday in November-December 2006, and did not take any malaria chemoprophylaxis. Staying in a coastal area in Goa, she travelled for three days to an area 300 km inland. She developed symptoms the day before her return to Sweden. On her return she visited her general practitioner twice, and a diagnosis of bronchitis was made and treatment with amoxicillin/clavulanic acid started. The patient was admitted to hospital with a fever of 40° C, hypotensive thrombocytopenia and hyponatraemia, diagnosis with *P. falciparum malaria* and a 1.5% parasitaemia, 16 days after her return. Malaria treatment was initiated with quinine and proguanil/atovaquone. Her condition gradually improved and she was discharged from hospital a week later.

**Denmark case 1**
A Dane in his 60s visited Goa for a two week holiday in December 2006. He stayed in Goa but visited a waterfall in an area east of Goa. His hotel accommodation in Goa had no air conditioning and no mosquito net. In accordance with the Danish travel recommendations at the time [3] he did not take any malaria chemoprophylaxis. He developed symptoms of fever and headache nine days after his return to Denmark, and was admitted to a local hospital three days later. Two days later, he was diagnosed with P. falciparum malaria, 0.3 % parasitaemia, and transferred to an infectious disease ward. On admission he had eye muscle paresis but with a normal cerebral magnetic resonance imaging scan, and thrombocytopenia. Because of these complications, he was started on intravenous quinine followed by mefloquine, and made an uneventful recovery.

**Denmark case 2**
A Dane in his 20s travelled to Goa for a two week holiday in November 2006, and stayed in a tourist hotel. He did not take any chemoprophylaxis. His symptoms developed in early December, one week after his return to Denmark, and he was admitted to hospital three days later with a low grade fever. Microscopy revealed a parasitaemia of 1%. He was treated with mefloquine with rapid clinical response and cleared his parasitaemia uneventfully.

**Denmark cases 3 and 4**
Two men in their 20s travelled together to India for three weeks from mid October to the beginning of November 2006. They visited Punjab, Goa, Delhi and Mumbai. Both took chloroquine tablets only. The Danish Statens Serum Institut considers chloroquine-only prophylaxis to be insufficient for malarious areas in India and recommends taking both chloroquine and proguanil [3]. Both men developed fever, headache and gastrointestinal symptoms on the last day of their holiday. One of the patients was diagnosed that day with *P. falciparum malaria* by microscopy and was successfully treated with proguanil/atovaquone (four tablets per day for three days). The diagnosis was confirmed by Binax NOW antigen test. The second patient initially tested negative by microscopy for malaria on three consecutive days while still taking chloroquine, but his blood tested positive on six weeks after onset of symptoms, and he was successfully treated with proguanil/atovaquone.

**Comment from TropNetEurop**
Goa, a popular winter resort for European tourists who visit beach hotels and resorts along the coast, has previously been considered to be very low-risk for malaria infection for travellers. It appears that this cluster of cases of imported falciparum malaria, acquired in the area to the north of Panaji, the capital of Goa, has coincided with a period of intense rainfall (50% above average) in the Goan and Konokan region since October 2006 [4]. This may be the cause of increased vector breeding and transmission during the current rainy season.

:he risk of infection will presumably decline with the coming dry season. Currently, however, there may be ncreased risk of malaria transmission and therefore all travellers who intend to visit this region of India are advised o use mosquito bite avoidance measures. Visitors may consider using WHO type IV prevention [5], which is nosquito bite prevention plus chemoprophylaxis with atovaquone/proguanil, doxycycline, or mefloquine, or they nay consider travelling with emergency standby treatment. Anyone who becomes unwell while on holiday or shortly fter their return should seek medical attention promptly. Falciparum malaria can be a life-threatening illness. The liagnosis can only be made if a careful travel history is taken, and testing done early, even for regions where malaria s not normally recognised. The risk in Goa will be monitored and we expect transmission to cease as the rainy eason ends in early 2007.

**Changes made in recommendations for travellers in some EU member states**

n December 2006, Denmark's Statens Serum Institut changed its malaria advice until further notice, as a precautionary measure. Until there is information on possible chloroquine resistance, the new recommendation for Danish travellers to Goa is type IV chemoprophylaxis, as described above [3]. Previously no chemoprophylaxis was advised.

n Sweden, no changes in the general recommendations for travellers have been made [2] but the Swedish Institute or Infectious Disease Control has informed about the Goa cases on their home page and sent a letter to all lepartments of Infectious Diseases and Infectious Disease Control. In this letter it is stated that: "Until we know more bout the present malaria situation, malaria prophylaxis can be considered for travellers visiting Goa. The drugs that night be used are Malarone, Lariam (mefloquine) or doxycycline". In addition, there have been several news sp on this topic on the national television.

No changes in the general recommendations for travellers have been made in Germany.

n addition, the recent cluster of malaria cases imported from Goa has also prompted the Advisory Committee for Malaria Prevention in United Kingdom Travellers (ACMP) to issue temporary change to its recommendations. The ACMP advises that travel advisors should highlight the risk of malaria, instruct on the use of mosquito bite avoidance neasures [6], and recommend malaria chemoprophylaxis to those travellers who will be visiting Goa, particularly reas north of Panaji, and who will be remote from medical care. This advice remains in effect until further notice as he situation is clarified. The recommended chemoprophylaxis is chloroquine plus proguanil. Alternatives are nefloquine, atovaquone plus proguanil, or doxycycline.

*Acknowledgements:*
*Thomas Zoller Charite hospital, Berlin, Germany; Johan Iversen, Department of Infectious Diseases, Hvidovre Hospital, Denmark; Toke Barfod, Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark;*

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back to top

別紙様式第2-1
番号16

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年3月5日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
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| 一般的名称 | 乾燥人フィブリノゲン | | 研究報告の公表状況 | ProMED/20070223.0668 | 公表国<br>アメリカ | |
| 販売名（企業名） | フィブリノゲンHT-Wf （ベネシス） | | | | | |

**研究報告の概要**

米国の科学者は、ヒトのブタ髄膜炎の北米での最初の症例を報告した。この患者は、髄膜炎で入院した以前には健康であった59歳のニューヨーク州北部在住の農夫である。この病院の予備的試験により、ブタに多い細菌で、髄膜炎を引き起こすStreptococcus suisの存在が明らかになった。Cornell 獣医薬大学の研究助手であり且つ獣医であるRuth Zadoks氏は、農夫と彼の農場のブタに見付かったStreptococcus suis の DNA レベルの分析結果を示した。農夫の細菌培養とブタのサンプルによって、Zadoks氏は、この患者がStreptococcus suisに実際に感染していると確認した。
Streptococcus suisは、過去20年間ヨーロッパにおいて時々、ヒトに感染した。しかし、人々が常に罹っていたのは東南アジア、特に中国であり、そこでは、2005年のアウトブレイクによって204人が感染し、38人が亡くなった。2005年には、南アメリカでも、アルゼンチンの最初の症例の報告があった。

**使用上の注意記載状況・その他参考事項等**

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分から人フィブリノゲンを濃縮・精製した製剤であり、ウイルス不活化を目的として、製造工程においてリン酸トリ-n-ブチル(TNBP)／ポリソルベート80処理、濾過膜処理(ナノフィルトレーション)、凍結乾燥の後、60℃、72時間の加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ヒトのブタ髄膜炎の北米での最初の症例報告である。<br>Streptococcus suisはブタレンサ球菌で、直径0.5～1.0μmの球状細菌である。万一、原料血漿にStreptococcus suisが混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

Archive Number 20070223.0668
Published Date 23-FEB-2007
Subject PRO/AH/EDR> Streptococcus suis, porcine, human - USA (NY)

STREPTOCOCCUS SUIS, PORCINE, HUMAN - USA (NEW YORK)
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A ProMED-mail post
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International Society for Infectious Diseases
<http://www.isid.org>

Date: Wed 21 Feb 2007
From: ProMED-mail <promed@promedmail.org>
Source: PigProgress.net [edited]
<http://www.pigprogress.net/ts_pi/pigprogress.portal/enc/_nfpb/true/tspi_portlet_news_singleeditorschoice1_3_actionOverride/__2Fportlets__2Fts__2Fge__2Fnews_singleeditorschoice1__2Fcontent__2FshowDetailsList/_windowLabel/tspi_portlet_news_singleeditorschoice1_3/tspi_portlet_news_singleeditorschoice1_3id/12716/_desktopLabel/pigprogress/_pageLabel/tspi_page_news_content/>

US scientists have confirmed the 1st reported case of pig meningitis in a human being in North America. The patient is a previously healthy 59-year-old farmer from upstate New York who was hospitalized with meningitis. The hospital's preliminary tests revealed the presence of _Streptococcus suis_, a bacterium prevalent in pigs, which can lead to meningitis.

"This should heighten awareness of the possibility of this illness for people with the occupation of dealing with swine," said Kara Willenburg, MD, of Bassett Hospital. "But it should not affect the lives of the general public."

Farmers can contract the disease from handling pigs and their feces. Butchers are also at risk. Washing hands and cooking pork thoroughly can minimize the risk.

Ruth Zadoks, research associate and veterinarian at Cornell University's College of Veterinary Medicine, provided the insights and a DNA-level analysis of _S. suis_ strains found in both the farmer and the pigs from his farm. With the farmer's bacterial cultures and the pig samples, Zadoks confirmed that the patient was indeed infected with _S. suis_.

"Even though we did not match the human strain with those of the pigs, the strain from the farmer matches superficially a strain found in both pigs and humans in Denmark and the Netherlands," said Zadoks. "Some strains are more likely to cause disease in humans."

_S. suis_ has occasionally affected humans in Europe over the last 20 years. However, people have regularly contracted the disease in

Southeast Asia, especially China, where a 2005 outbreak resulted in 204 human cases with 38 deaths, and some 600 pigs killed. In 2005, South America also documented its 1st case from Argentina.

"It could be that the disease is spreading," said Zadoks. "But also we may be just better at recognizing it."

All human cases of _S. suis_ have originated with an animal infecting a human. Public health officials have no fears that the pig bacteria will spread from one human to another.

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[This case represents the more classic meningitis presentation of human _S. suis_ infection. Although it is stated that this case was the 1st in North America, a 2002 paper (Marie J, Morvan H, Berthelot-Herault F, et al: Antimicrobial susceptibility of _Streptococcus suis_ isolated from swine in France and from humans in different countries between 1996 and 2000. J Antimicrob Chemotherap 2002;50: 201-209 available at <http://jac.oxfordjournals.org/cgi/content/full/50/2/201>) discusses 4 human isolates of _S. suis_ from Canada.

In addition, the University of Iowa website (<http://www.public-health.uiowa.edu/CEID/Suis.htm>) regarding this organism states, "The bacterium has caused sporadic illness in other countries as well, including Canada, and a human case of infection with the bacterium has recently been described in the USA. Several investigators have suggested this low number of cases in the USA is due to under- or misdiagnosis, rather than a true absence of this disease in the human population." It is possible that the site is referring to the New York case.

The 2005 Chinese outbreak was atypical because most previous cases had been sporadic, not in an outbreak, and cases of streptococcal toxic shock syndrome (STSS) occurred. The outbreak was summarized in Yu H, Jing H, Chen Z, et al: Emerg Infect Dis 2006;12: 914-20 <http://www.cdc.gov/ncidod/eid/vol12no06/05-1194.htm>. Part of the discussion (with citations removed) is found below:

Beginning mid-Jun 2005, a major outbreak of _S. suis_ killed 647 pigs in almost the same areas as the outbreak in humans. The outbreak in swine peaked around 20 Jul 2005 with about 4 dead pigs in each affected village. _S. suis_ caused 98 percent of the deaths of these pigs in Sichuan during this period. All infected pigs came from backyard farms, usually with one sick pig in the herd. A pathogenic strain could have spread by distributing infected piglets to the backyard pig farms and then propagated among healthy pigs. The single ribotype identified in the investigation lends support to this theory.

The main risk factor for _S. suis_ infection in the outbreak was direct involvement in slaughtering sick pigs and preparing carcasses

of pigs that died of unknown causes. Unlike professionals in modern abattoirs, the local farmers did not wear protective gear or gloves. Normally one or 2 persons carried out the procedure, which involved bloodletting though a neck artery, manually inflating the carcasses, scalding the pigskin with about 80 deg. C water, and splitting and shaving the skin with large knives. Scalding and shaving were often performed together. The farmers then sliced the meat into smaller pieces before cooking for food. The complete process of slaughtering could take more than one hour. Our study demonstrated that all patients had been infected during direct contact with blood or tissues of sick or dead pigs. Often, this may have occurred through direct exposure of skin wounds. Droplet exposure may also have occurred during slaughter or processing of carcasses, but we could not document this occurrence. The observed risk factors were consistent with those reported in other studies. No evidence of infection from eating cooked pork from these pigs was observed. The uncooked meat was shared with neighboring families, but these villagers normally do not eat raw meat or raw animal viscera. Person-to-person transmission was highly unlikely since we found no disease in family members, neighbors, or healthcare workers who had not been exposed to sick or dead pigs.

One missing link in the outbreak is the exact relationship between a dead goat in the early cluster of patients and the subsequent propagation of the _S. suis_ infections. We could not confirm these 2 cases microbiologically. We speculate that _S. suis_ caused these 2 early human infections because they occurred shortly after exposure to the dead goat, and because clinical manifestations in the 2 patients were similar to those of others in the outbreak. Human _S. suis_ infection after exposure to sick goats has not been reported, despite isolation of the organism from these animals. In backyard farms where different animals are kept together, _S. suis_ infection could have been transmitted between pigs and goats. Animal surveillance would help establish the role of animals other than pigs in carriage of the bacteria and the potential for causing human infections.

Clinically, 3 distinctive forms of human _S. suis_ infection occurred, namely, STSS, sepsis, and meningitis. STSS has not been reported from _S. suis_ infection, although it has been previously described in other streptococcal infections and [a similar illness from] _Staphylococcus aureus_ infections. Unusual STSS-like illnesses brought the outbreak to the attention of local health authorities. A dose effect may explain the relatively high proportion of STSS in this outbreak. While other explanations like co morbid conditions, e.g., asplenia, diabetes mellitus, alcoholism, and malignancy, have been reported, this was not the case for the outbreak in our study, which involved previously healthy adults.

Laboratory examination confirmed virulence factors in the _S. suis_ isolates from this outbreak. They include mrp, sly, and ef, although their precise clinical role has not yet been shown. These isolates (mrp+, ef+, sly+) are related to European strains that are considered to be more virulent than North American strains. Genome analysis could determine if novel virulence genes were involved. - Mod.LL]

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[see also:
2006
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Streptococcus suis, porcine, human, 2005 - China (Sichuan) 20060411.1082
2005
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Streptococcus suis, porcine, human - China 20050730.2217
Streptococcus suis, human - China (Hong Kong) 20050728.2196
Streptococcus suis, porcine, human - China (Sichuan) (02) 20050728.2194
Streptococcus suis, porcine, human - China (Sichuan) 20050727.2179
Undiagnosed deaths - China (Sichuan) (05): Strep. suis susp. 20050726.2169
Undiagnosed deaths - China (Sichuan): RFI 20050724.2131]
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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2006年12月25日 | 新医薬品等の区分 | 厚生労働省処理欄 |
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| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | AABB WEEKLY REPORT 2006; 12(44): 4-5 | 公表国<br>アメリカ |
| 販売名<br>(企業名) | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④コンコエイトーHT（ベネシス）<br>⑤クリスマシンーM（ベネシス） | | | |

**研究報告の概要**

伝達性海綿状脳症（TSE）諮問委員会が2006年12月15日に公開で開催され、ヒト血漿由来抗血友病因子製剤におけるvCJDの感染リスクについて討議した。この諮問委員会では以下の2つのトピックを取り上げた。
1)ヒト血漿由来抗血友病因子製剤（FVIII）におけるvCJDへの曝露についてのFDAのリスク評価についての検討及びコミュニケーション手段
2)血漿由来FVIII製造におけるTSEクリアランスのレベル
このリスク評価に対して諮問委員会は、報告が強制でないことから過小報告をFDAは如何に説明しようと考えているのか、及び最終製品が4 logのリダクションを有しているとの仮定をするのにFDAはどんな具体的なエビデンスを用いたかについて懸念を表明した。委員会はまた、感染性病原体のクリアランスを決定するためのステップとしてスパイク実験を用いることに支持を表明した。
提示されたリスク伝達の計画について長い議論が交され、委員会の考えは理に適ったものであるが、改善の余地があるとの結論に至った。委員会は型通り全てに適合する哲学はありえないことに合意した。
ミニマムTSE病原体リダクション・ファクターの有効性についてのFDAからの助言求めに応じて、PPTAの病原体安全対策委員会の議長が製造工程で除去されたプリオンのレベルについてのデータを示したが、値は企業によって3.9から>9.05 logまでの範囲があった。このプレゼンテーションには、検体調製の方法論からlogリダクションが工程全体で如何に決定されたかまでの話題について質問が殺到し、最終的に外因性物質を用いた実験は血漿由来第VIII因子製剤の安全性を保証する一手段であるとの多数決での決定に至った。しかし諮問委員会は、FDAに対して何が適切なlogリダクション・ファクターかについての勧告をすることができなかった。

| 報告企業の意見 | 今後の対応 |
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| 2006年12月15日開催の米国伝達性海綿状脳症委員会（TSEAC）会合において、抗血友病因子製剤のvCJDの感染リスクについての検討結果についての報告である。(BENE2006-025の結果の報告)<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wfの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

In an effort to underscore the overall value of the proposed trial, Daniel Freilich, MD, CRD, MC, the lead investigator in the proposed Navy trial, pointed out that traumatic hemorrhagic shock is a significant public health problem that currently sees an estimated 50 percent mortality rate. "The current treatment is unsatisfactory," he said, noting that preclinical data show a strong prospect for patient benefit.

When questioned by some committee members on the homogeneity of the trial design, Freilich pointed out that although socioeconomic and other demographic data are not readily available, trauma patients whose transport to a hospital is less than approximately 15 minutes would not be included in the trial, which would help to avoid disproportionate emphasis on certain populations. Supporters of the study also say that another way to ensure equitable distribution of risk would be to go beyond the urban setting and include rural trauma centers in the trial. Nonetheless, some critics have said that the proposed trial design is unethical and that FDA's previously cited reasons for blocking the study remain valid.

In reviewing the trial design for FDA, Laurence Landow, MD, FRCPC, medical officer in the Division of Hematology in FDA's Office of Blood Research and Review, reiterated the agency's concerns about safety and dosing. FDA is concerned that the number of severe adverse events observed in HBOC clinical trials with animals could be even higher in the critically ill trauma patient population. The agency also believes that minimal evidence has been provided to support the safety of the default HBOC infusion rate.

One of the major roadblocks BioPure has faced is the lack of Phase II data that would support a Phase III trial. The multicenter, randomized Phase II trial involved individuals undergoing nonemergency orthopedic surgery. Of the 353 patients who received HBOC 201, 10 patients died and 21 suffered heart complications whereas six people from the control group died and five experienced heart problems. The Phase II trial did not include trauma patients, the population that would be evaluated according to the design of the proposed Phase III trial. The committee discussed the possibility that BioPure could plan a smaller Phase II trial that more closely resembles the intended use of the product rather than have FDA approve the Phase III clinical trial sought by the Navy.

If FDA agrees with BPAC's recommendations, it would mark the fourth time since June 2005 that the agency has blocked Hemopure trials from taking place.

## TSE Advisory Committee Convenes to Discuss Plasma-derived Factor VIII Safety

The Transmissible Spongiform Encephalopathies (TSE) Advisory Committee held an open public meeting Dec. 15, 2006, in Silver Spring, Md., to discuss the potential risk of variant Creutzfeldt-Jakob disease (vCJD) in human plasma-derived antihemophilic factor products. The advisory committee had two topics for discussion: 1) review of FDA's risk assessment for potential exposure to vCJD in human plasma-derived antihemophilic (FVIII) products and communications materials; and 2) the levels of TSE clearance in the manufacture of plasma-derived FVIII.

FDA presented a risk assessment to the TSE Advisory Committee to seek its advice on key message points in both the risk assessment itself and appropriate ways to communicate this information to physicians, patients and the general public.


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In response to the risk assessment — which found that the potential to transmit vCJD from plasma-derived FVIII products used by some patients with hemophilia A and von Willebrand disease is highly uncertain, but appears likely to be very low — the advisory committee voiced concerns about how the FDA was going to account for underreporting since reporting is not mandatory and what objective evidence FDA used in making its assumption that there is a four-log reduction in the final product. The committee also expressed its support for the use of spiking experiments as a step in the right direction for determining the clearance of the infectious agent.

A lengthy discussion was held on the proposed risk communication plan, with the committee concluding that the concept was sound but that there was some room for improvement. The advisory committee agreed that there cannot be a "one size fits all" philosophy. The key points for a physician would be different from those for a patient. For example, the message to physicians should be that infected patients need to receive the same standard of care as noninfected patients, not be treated differently as when HIV was first an issue during the 1980s. For patients, there should be a focus on the positive aspects of the therapy, i.e., the benefits of taking the product.

The committee also addressed the safety and efficacy of plasma-derived FVIII products. In response to an FDA request for advice on the efficacy of a minimum TSE agent reduction factor — which has been demonstrated in laboratory-based experimental models to enhance vCJD safety of the products — the chair of the Plasma Protein Therapeutics Association (PPTA) Pathogen Safety Steering Committee presented data on the level of the prion removed by the manufacturing process, a value that ranged from 3.9 to >9.05 logs, depending on the company. The presentations prompted questions on subjects ranging from the methodology of preparing the samples to how the log reduction was determined for the overall process, ultimately leading to a nonunanimous decision that, based on the scientific evidence, exogenous studies are a means of assuring the safety of plasma-derived FVIII products. However, the advisory committee was unable to make a recommendation to FDA for what would be an appropriate log reduction factor.

## AABB Bulletin

# New *AABB Weekly Report* to Launch in January

*AABB Weekly Report* will be replaced in January by a new e-newsletter that will bring more timely information about the latest developments affecting the transfusion medicine and cellular therapies fields to all AABB members as part of their membership dues.

Brief articles highlighting what has occurred in the field during the previous week will be included in the soon-to-be launched publication. The articles — on topics ranging from regulatory issues to standards and accreditation — will link to more detailed content on the Web. Links to new government Web resources also will be included in this e-newsletter. Periodic news flashes will be distributed if there is urgent news to communicate to members.

News reported by the mainstream media will continue to appear daily in *AABB SmartBrief*, which launched in October. Available to both members and nonmembers free of charge, *AABB SmartBrief* summarizes the day's most important news — selected from a wide range of media sources — providing links to the original articles for those who want to learn more about a particular topic. The content is organized into categories — science and health, government developments and emerging


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No. 20

# 医薬品 研究報告 調査報告書

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**研究報告の概要**

○FDAの専門家委員会が血漿分画製剤におけるvCJDリスクについて討議

血漿由来の第Ⅷ因子製剤(pdFVIII)による患者への変異型クロイツフェルト・ヤコブ病の病原因子伝播のリスクは、極めて低いと見られる。生物製剤評価調査センター(CBER)のSteven Anderson博士は、「しかし、リスクはゼロではない」と伝達性海綿状脳症(TSE)諮問委員会で話している。CBERは、2005年10月31日の委員会で提示されたコンピュータモデルと仮説に基づいたリスク分析案の概要を示した。入力された情報は、英国におけるvCJDの推定発生率、渡航歴による供血延期の効果、動物モデルでの血液中の感染性の強さ、伝播の効率性、製造工程における除去、患者によるFVIIIの使用量を含んでいる。Anderson博士は、リスク分析には不確定な要素が含まれていると強調した。このモデルでは、現在米国で実施されている供血延期方針の有効性は85～99%と見積もられている。重要度解析では、リスクを決定する主要な要素は、製造工程におけるvCJD感染因子の低減である。

他にも幾つかの研究が報告され、委員会では、「これらの研究は見当違いという訳ではなく、他に方法がない以上継続する必要がある。しかし、血液との関連は未知である」と結論づけた。こうした懸念にもかかわらず、TSE減少を決定する根拠となる外部スパイク物質の研究を行うかどうかの採決は、免疫アッセイだけではなくバイオアッセイを行うという条件で可決された。

283

| 報告企業の意見 | 今後の対応 |
|---|---|
| FDAの専門家会議が第Ⅷ因子製剤のvCJDのリスクについて討議したとの報告である。 | これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してvCJDが伝播するという証拠はない。異常プリオンがアルブン製剤の製造工程で効果的に除去されるとの報告もあるが、輸血によりvCJDに感染する可能性が示唆されたことから、今後も情報の収集に努める。尚、日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。 |

**使用上の注意記載状況・その他参考事項等**

赤十字アルブミン20
赤十字アルブミン25

血液を原料とすることに由来する感染症伝播等

39

## Scottish Decision to Use Chagas' Test Frustrates Some Donors

The National Blood Transfusion Service for England and Wales has chosen to perform Chagas' testing on donors at risk for Chagas'. But the Scottish National Blood Transfusion Service (SNBTS) has decided it is too expensive to perform testing for the estimated 200 people at risk for the disease and too complicated to obtain testing results for people who have been tested in Wales and England by the National Blood Transfusion Service.

The different UK policies have frustrated at least one long-time donor. In an interview with *The Scotsman* (12/12/06), Michael Turnbull, whose mother is from Brazil, said he was allowed to donate blood in England after being tested, but has been barred from giving blood in Scotland. Mr. Turnbull, who has type O-negative blood, says he feels so strongly about donating that he plans to give blood when visiting his daughter in Bedford, England.

Jack Gillon, MD, a consultant with the SNBTS, told *The Scotsman*: "We have relatively few donors in this category now, and on the whole the resources issue is a real one. Setting up a test with all the safeguards and quality assurance you need is quite risk intensive... We are not in a position where we can take that lightly, but we have to use the resources we have got to the best of our ability." ♦
The blood transfusion services in England, Wales and Scotland currently defer donors who were born in South America or Central America (including Southern Mexico) or whose mothers were born in these regions; who were transfused in South America, Central America (including Mexico); or who lived and/or worked in rural subsistence farming communities in these countries for a continuous period of four weeks or more.

For situations other than transfusion, blood services may accept a donor if it has been at least six months from the date of the last exposure, and a validated test for *T. cruzi* antibody is negative.

## FDA Expert Panel Addresses Risk of vCJD in Plasma Products

The risk of transmission of the variant Creutzfeldt-Jakob disease (vCJD) agent to recipients of plasma derived Factor VIII (pdFVIII) manufactured in the US most likely is extremely small. "But the risk may not be zero," Steven Anderson, PhD, from the Center for Biologics Evaluation and Research's (CBER) Office of Biostatistics and Epidemiology told the Transmissible Spongiform Encephalopathies Advisory Committee at its meeting today.

CBER staff briefed the panel on a draft risk assessment based on a computer model and assumptions that had been presented to the committee on October 31, 2005. The inputs included prevalence estimates of vCJD in the UK, the efficiency of donor deferrals for travel, the quantity of infectivity in blood based on animal models, the efficiency of transmission, clearance during manufacturing, and FVIII usage by the patient population.

Dr. Anderson emphasized that the risk assessment contained significant uncertainty. For instance, the prevalence estimates varied from 1 in 1.8 million for UK epidemiologic studies that predicted 70 cases of vCJD between 2002 and 2080, to 237 per million derived from tonsil/appendix tissue surveillance (3 positives in 12,674 samples tested). The model assumes that the efficacy of the currently applied donor

(continued on page 6)

Risk of vCJD in Plasma Products (continued from page 5)

Deferral policies in the US is 85-99 percent. According to an importance analysis, the major factor determining risk is the log reduction of vCJD agent achieved by the manufacturing process.

> **"It is important to avoid complacency and retain a sense of proportion on this particular issue."**
>
> ***– Mark Skinner***

Dorothy Scott, MD, from OBRR's Division of Hematology, reported that a preliminary review of clearance studies performed by the plasma industry indicated that all products in the US market achieve a clearance of at least 4-6 logs in spiking experiments. Dr. Anderson estimated that the individual vCJD risk for a severe Hemophilia A patient is 1 in 1.3 million if the lower prevalence estimate is considered and 1 in 15,000 if the highest prevalence estimate is used. The estimated risk for that same population is 1 vCJD infection in 3,077 years for the lower prevalence, and 1 vCJD infection in 35 years for the highest prevalence.

Mark Skinner, president of the World Federation of Hemophilia welcomed that FDA analysis. But he emphasized that "it is important to avoid complacency and retain a sense of proportion to this particular issue." His advice was to maintain timely communication with openness and transparency, acknowledgement of areas of uncertainty and avoidance of patient stigmatization with consequent denial of access to care. Stigmatization was also the theme of Jan Hamilton from the Hemophilia Federation of America and Val Bias, from the National Hemophilia Foundation.

The Committee of Ten Thousand (COTT), represented by Richard Colvin, MD, PhD also welcomed the assessment but was skeptical of some of the assumptions. COTT President Cori Dubin pleaded for a compensation system for injured recipients. Dave Cavenaugh, also from COTT, raised substantial doubts about surveillance for bovine spongiform encephalopathy in the US, was very critical of the Department of Agriculture, and said he still believes that vCJD is a clear and present danger for recipients of plasma derived clotting factors.

In the second part of the TSEAC meeting, Thomas R. Kreil, PhD, chair of the Plasma Protein Therapeutics Association Pathogen Safety Steering Committee presented the current status of TSE clearance studies being performed by the industry. His presentation generated substantial discussion about the nature of the spiking materials used in the studies and the measurement of clearance based on immunological methods.

The committee concluded that "these studies are not irrelevant and should be continued because there is nothing else available. However, the relationship with blood is unknown." Despite these concerns, when asked specifically whether studies with exogenous spiking materials could be the basis for the determination of a TSE reduction factor, the committee voted yes (10-0 with two abstentions), provided that the assessment is based on bioassays, not just immunological assays.

FDA has prepared a wealth of resources communicating the results of the risk assessment to the patient community, physicians, and the population in general. The draft risk assessment and the draft communication materials are posted on the FDA Web site at www.fda.gov/ohrms/dockets/ac/06/briefing/2006-4271b1-index.htm ♦

別紙様式第2-1

No. 21

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2007. 2. 26 | 該当なし | |

| 一般的名称 | 乾燥濃縮人血液凝固第Ⅷ因子 | 研究報告の公表状況 | ABC News Letter. 2007 Feb 9; 7. | 公表国 |
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| 販売名(企業名) | クロスエイトM250(日本赤十字社)<br>クロスエイトM500(日本赤十字社)<br>クロスエイトM1000(日本赤十字社) | | | 英国 |

**研究報告の概要**

○輸血がvCJD二次的感染拡大を引き起こすことはなさそうである
将来のvCJDによる死亡率は、供血に関する公衆衛生上の施策によって予想されていたよりも遙かに低くなるだろうと英国の研究者が報告した。Royal Society Journal Interface誌オンライン版によると、ロンドン衛生・熱帯病教室と全国CJDサーベイランスユニットは、2080年までの輸血によるvCJDの死亡例は50例と予測した。感染牛の摂食によるvCJD感染が排除されたため、現在では輸血による伝播が最も可能性が高いと研究者は話している。
この研究では、数学モデルを使用して輸血によるvCJDの感染拡大が発生した場合のシナリオを試算し、公衆衛生上の施策の影響も分析した。vCJD感染リスクに対応して、英国では1980年以降輸血歴のある人からの供血禁止、白血球の除去、血漿分画製剤製造のための血漿輸入など様々な方策を実施してきた。研究者は、「こういった安全対策がない場合、感染例は2080年までに900例になるが、生物学的には起こりそうもない。楽観的な想定では、公衆衛生上の施策によって感染は250例まで減少し、さらに生物学的可能性のあるシナリオが検討の対象となる。この研究は、大規模で持続的な感染拡大という事態は、可能性はあるが起こりそうもなく、公衆衛生上の施策が効果的であるという考えを支持するものである」と記している。
研究者によると、若い人が供血し60代以上の老人に輸血されることが多いため、vCJDは若い人から老人へと伝播するが、受血者は高齢で供血することは少なく、感染伝播したとしてもvCJDが供血システムに戻ることはないと考えられる。このことは、政府による他の施策と合わせて、輸血によるvCJDの死亡症例数を減少させる。一つ不明な点は、外科手術用器具を介しての危険性である。これには「年齢による防御効果」がなく、汚染防止の対策もリスクを完全に排除できない。しかし、汚染された手術器具への暴露後のvCJD発症の証拠はない。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 供血に関する公衆衛生上の施策によってvCJDによる死亡率は予想されていたよりも遙かに低くなると考えられ、英国における2080年までの輸血によるvCJDの死亡例は50例と予測されるとの報告である。 | これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してvCJDが伝播するという証拠はない。異常プリオンが本製剤の製造工程で効果的に除去されるとの報告もあるが、輸血によりvCJDに感染する可能性が示唆されたことから、今後も情報の収集に努める。尚、日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、英国を含む欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980年～1996年に1日以上の英国滞在歴のある方からの献血を制限している。 |

**使用上の注意記載状況・その他参考事項等**

クロスエイトM250
クロスエイトM500
クロスエイトM1000

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

Australian Red Cross to Screen Platelets for Bacterial Contamination (continued from page 6)

A Red Cross spokesman called implementing bacterial detection a "vitally important measure" that demands immediate attention. "To assist governments in their decision making when they meet at the end of March, the ARCBS Board has decided to absorb the one-off capital costs in 2006-07 in the interests of the enhanced safety of the Australian community," she said. "This will be achieved without any diminution in our capacity to meet the ongoing demand for blood."

**"When new technologies are available to make it better and are recommended by the experts in that area, then really it's incumbent upon government to ensure we continue to get the safest possible [blood] supply."**

***– Australian Medical Association President Mukesh Haikerwal, MD***

Under the new plan, the Red Cross would screen 100 percent of platelets by April next year. Current laws require only 5 percent to be checked. If left to the scheduled budgetary processes, the Red Cross believes full screening will not be possible until 2009, placing more Australians at risk.

Australian Medical Association President Mukesh Haikerwal, MD told *The Age* that health politicians needed to act quickly on the advice of their blood experts. "A very important part of our surgical and medical care is our ability to provide blood products that are safe and disease free," he said. "When new technologies are available to make it better and are recommended by the experts in that area, then really it's incumbent upon government to ensure we continue to get the safest possible supply." ♦

## Blood Transfusion Unlikely to Create Secondary vCJD Epidemic

The future death toll from vCJD is likely to be far lower than originally anticipated because public health interventions have dramatically reduced the number of infections that could have been transmitted by blood transfusion, British researchers reported this week. In a paper published online in the *Royal Society Journal Interface*, investigators at the London School of Hygiene & Tropical Medicine and the National CJD Surveillance Unit, predicted just 50 deaths from transfusion-transmitted vCJD by 2080.

A large scale epidemic of vCJD via blood transfusion running into thousands of cases is unlikely to occur, and public health interventions, such as a ban on donation by transfusion recipients, were timely and effective in limiting the scale of the epidemic, the researchers said.

According to the latest UK Department of Health statistics, there have been 112 vCJD deaths in the UK in which the diagnosis has been pathologically confirmed through post mortem examination of brain tissue. Since the primary method of contracting vCJD – eating infected meat – has been effectively eliminated, blood transfusions now are considered to be the most likely way of transmission, the researchers said.

In 2004, a study of 13,000 appendix and tonsil samples suggested that thousands of people might unknowingly be harboring vCJD. The discovery of three individuals suspected to have contracted variant Creutzfeldt-Jakob disease (vCJD) through blood transfusions heightened concerns that a secondary epidemic via human-to-human transmission could occur in the UK.

Since that time, however, only four people have been shown to have contracted vCJD through blood transfusions. They were among a group of 66 who received blood transfusions from donors who went on to develop the disease.

(continued on page 8)

## On Believing in Prions

"It's not very politically correct if you don't believe in prions. It means that you won't get any grant money.

– *Yale University neuropathologist Laura Manuelidis, MD, in a recent interview with* Nature *on the controversy over her contention that a virus causes neurological conditions such as Creutzfeldt-Jakob disease in humans and scrapie in animals, rather than an infectious protein, or 'prion,' as most researchers believe.*

Transfusion-Transmitted vCJD Epidemic Unlikely (continued from page 7)

The researchers used mathematical models to explore scenarios in which a new vCJD epidemic, carried by infected blood, might develop. They also assessed the impact of public health interventions that have been implemented on its spread.

To address the risk of vCJD infection, the British government and the National Blood Service put in place a number of measures to protect transfusion recipients from contracting vCJD, the researchers pointed out. These include prohibiting anyone who has received a blood transfusion since 1980 from donating blood. In addition, since 1999, white blood cells – which are the most likely to carry the infection – have been removed from blood used for transfusions. Blood derivatives now only manufactured using plasma from the US.

Without these blood safety measures, the researchers wrote, "the size of the epidemic (up until 2080) was bounded above by 900 cases, with self-sustaining epidemics also possible; but the scenarios under which such epidemics could arise were found to be biologically implausible." They continued: "Under optimistic assumptions, public health interventions reduced the upper bound to 250 and further still when only biologically plausible scenarios were considered. Our results support the belief that scenarios leading to large or self-sustaining epidemics are possible but unlikely, and that public health interventions were effective."

According to the researchers, young people – those in their 20s, 30s and 40s – are most likely to donate blood, but people aged 60 and over are most likely to need transfusions. This means it is possible for vCJD to be transferred from a young donor to an old recipient, they said. But, because of their age, these recipients would not go back to donate blood so even if they were infected through transfusion, the disease would not be passed back into the blood donor system. This fact, along with other interventions put in place by the government, limits the number likely to die from vCJD contracted through blood transfusions, they said.

"Patients requiring blood transfusions do really need them, often because they are in a life-threatening situation, so we hope this study will reassure people of the remote risk of contracting this disease," Azra Ghani, MD, an epidemiologist who worked on the study, said in an interview with BBC News.

But she added: "One uncertainty is around the dangers linked to surgical instruments, because there you would not have the 'age protection' factor, and we know that contamination protection measures do not remove the risk. "However there has been no evidence of a case of vCJD developing after exposure to contaminated instruments," she pointed out. (Sources: *Medical Laboratory World*, 2/7/07; BBC News, 2/7/07)

**Citation:** Clarke P *et al.* Is there the potential for an epidemic of variant Creutzfeldt-Jakob disease via blood transfusion in the UK? J R Soc Interface FirstCite Early Online Publishing, 1742-5689. ♦

別紙様式第2-1
番号8

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2006年12月8日 | 新医薬品等の区分 | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | CDC/vCJD/ 20061129 | 公表国<br>アメリカ | | |
| 販売名<br>（企業名） | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④コンコエイトーHT（ベネシス）<br>⑤クリスマシンーM（ベネシス） | | | | | |

**・研究報告の概要**

バージニア州保健局とCDCは、米国住民におけるvCJD確定症例を公表した。これは米国の住民において確認された3番目のvCJD症例である。この米国の最新の症例は、サウジアラビアで生まれて育ち、2005年終りから米国に居住した青年に発生した。この患者は2001年以来、一時的に最長3ヶ月間米国に時折滞在しており、1989年には短期の滞在の経験がある。2006年11月の終りになって、カリフォルニアサンフランシスコ記憶・老齢センターの臨床プリオン研究チームが、扁桃と脳生検組織についての病理学検査からvCJDの臨床診断を下した。米国住民における先に報告された2つのvCJD症例は、各々患者は英国で生まれ育ち、その地で患者らはその疾患の原因病原体に感染したと考えられている。
サウジアラビアではウシのBSEは1件も報告されていないが、英国からの汚染されたウシ製品が英国でBSEが流行している期間に長年、サウジアラビアに輸出されていた可能性がある。
この患者は、輸血を受けたことも、過去に神経外科的手術をしたことも、又ヨーロッパに滞在或いは訪問したこともなかった。この患者の病歴、サウジアラビアでBSE汚染のウシ製品を摂取したことより発生したとされるvCJD症例報告、及び食物関連のvCJDの潜伏期間が7年を超えるとの予想に基づき、この米国の症例は、サウジアラビアに住んでいた子供の時代に摂取した汚染されたウシ製品から感染した可能性が極めて高い。この患者は、供血歴はなく、公衆衛生調査によりこの患者から米国住民に感染するリスクのないことが確認された。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国で3例目のvCJD患者が確認されたが、生まれ育ったサウジアラビアで感染したものと推定され、米国民にvCJDを感染させるリスクもないことが確認されたとの報告である。<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wfの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

# vCJD (Variant Creutzfeldt-Jakob Disease)



**Confirmed Case of Variant Creutzfeldt Jakob Disease (vCJD) in the United States in a Patient from the Middle East**

The Virginia Department of Health and the Centers for Disease Control and Prevention announce the recent confirmation of a vCJD case in a U.S. resident. This is the third vCJD case identified in a U.S. resident. This latest U.S. case occurred in a young adult who was born and raised in Saudi Arabia and has lived in the United States since late 2005. The patient occasionally stayed in the United States for up to 3 months at a time since 2001 and there was a shorter visit in 1989. In late November 2006, the Clinical Prion Research Team at the University of California San Francisco Memory and Aging Center confirmed the vCJD clinical diagnosis by pathologic study of adenoid and brain biopsy tissues. The two previously reported vCJD case-patients in U.S. residents were each born and raised in the United Kingdom (U.K.), where they were believed to have been infected by the agent responsible for their disease. There is strong scientific evidence that the agent causing vCJD is the same agent that causes bovine spongiform encephalopathy (BSE, commonly known as mad cow disease).

Variant CJD is a rare, degenerative, fatal brain disorder that emerged in the United Kingdom in the mid-1990s. Although experience with this new disease is limited, evidence to date indicates that there has never been a case transmitted from person-to-person except through blood transfusion. Instead, the disease is thought to result primarily from consumption of cattle products contaminated with the BSE agent. Although no cases of BSE in cattle have been reported in Saudi Arabia, potentially contaminated cattle products from the United Kingdom may have been exported to Saudi Arabia for many years during the large U.K. BSE outbreak.

The current case-patient has no history of receipt of blood, a past neurosurgical procedure, or residing in or visiting countries of Europe. Based on the patient's history, the occurrence of a previously reported Saudi case of vCJD attributed to likely consumption of BSE-contaminated cattle products in Saudi Arabia, and the expected greater than 7 year incubation period for food-related vCJD, this U.S. case-patient was most likely infected from contaminated cattle products consumed as a child when living in Saudi Arabia (1). The current patient has no history of donating blood and the public health investigation has identified no risk of transmission to U.S. residents from this patient.

As of November 2006, 200 vCJD patients were reported world-wide, including 164 patients identified in the United Kingdom, 21 in France, 4 in the Republic of Ireland, 3 in the United States (including the present case-patient), 2 in the Netherlands and 1 each in Canada, Italy, Japan, Portugal, Saudi Arabia and Spain. Of the 200 reported vCJD patients, all except 10 of them (including the present case-patient) had resided either in the United Kingdom (170 cases) for over 6 months during the 1980-1996 period of the large UK BSE outbreak or alternatively in France (20 cases).

As reported in 2005 (1), the U.S. National Prion Disease Pathology Surveillance Center at Case Western Reserve University confirmed the diagnosis in the one previously identified case of vCJD in a Saudi resident. He was hospitalized in Saudi Arabia and his brain biopsy specimen was shipped to the United States for analysis. This earlier vCJD case-patient was believed to have contracted his fatal disease in Saudi Arabia (1).

1) Belay ED, Sejvar JJ, Shieh W-J, Wiersma ST, Zou W-Q, Gambetti P, Hunter S, Maddox RA, Crockett L, Zaki SR, Schonberger LB. Variant Creutzfeldt-Jakob disease death, United States. Emerg Infect Dis 2005, 11 (9):1351-1354.

Program Contents

- Topic Home
- Questions and Answer and vCJD
- Fact Sheet: vCJD
- Epidemiology
- Risk for Travelers
- Prevention
- Treatment
- News Archive
- References and Resou

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# 医薬品　研究報告　調査報告書

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| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 合衆国で製造されたヒト血漿由来第 VIII 因子製剤を投与されたことのある重度血友病 A 患者及び重度疾患のあるフォン・ウィルブランド病患者が vCJD 病原因子に暴露される確率と程度、及び vCJD に感染するリスクについて数量的な見積りを行った。評価の結果、米国で製造される血漿由来 FVIII 経由の vCJD 感染リスクは非常に低いと推定されるが、しかしゼロではないということが示唆された。モデルによる推定の結果より、vCJD 病原因子への暴露の可能性があり、非常に低いとはいえ潜在的感染リスクがあることが示唆されたが、全般的 vCJD リスク又は患者個々の実際のリスクについては、モデルにより「正確に」見積もることは不可能である。実際のリスクがどの程度であるのかは極めて不確かであるが、リスクの度合いを左右する最も重要な要因は、製造工程を通しての vCJD クリアランス、各患者の製剤使用量、及び英国供血者母集団における vCJD 有病率であることをリスク評価モデルは示している。製造工程による削減レベルが 4～6 $\log_{10}$ であると仮定した場合、モデルを用いた推定では、血漿由来 FVIII を投与されている重度血友病 A 患者 1 人 1 年当たりの潜在的リスクは、15,000 回に 1 回（高いほうの vCJD 推定有病率かつ製剤使用量が多量のとき）から 940 万回に 1 回（低いほうの vCJD 推定有病率かつ製剤使用量が少量のとき）の範囲である。<br>リスク評価の結果を検討する上で留意すべき重要なこととして、英国での提供血漿より製造された血漿由来製剤を長期に渡り投与されている患者に vCJD が発症したと広く世界に報告された例はなく、このことからも、血漿由来 FVIII からの実際の vCJD 感染リスクは非常に低いであろうと考えられた。しかし発症例がないことは、将来いつか、発病を来たすような感染源に患者が暴露される可能性を否定するものではないともされた。<br>詳細は添付資料の通り。 | 2.重要な基本的注意<br>(1)<br>2)<br>現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病(vCJD)等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| リスク評価においても示されている通り、血漿分画製剤による vCJD 伝播が疑われる症例は報告されていないこと、並びに、血漿分画製剤では分画精製におけるウイルス不活化／除去工程により、プリオンが不活化あるいは除去できると考えられ、プリオン病感染のリスクが十分に低減されると考えられることより、血漿分画製剤による vCJD 伝播の可能性は極めて小さいと考えている。 | 当該感染症に関し、引き続き、情報の収集を行っていく。<br>また、同様に同一生物種等から人に感染すると認められる疾病に関する情報の収集に努める |

感染症の用語は、MedDRA/J　version（10.0）を使用。

# DRAFT

# Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US

**November 27, 2006**

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# TABLE OF CONTENTS

EXECUTIVE SUMMARY....1
RISK ASSESSMENT
I. INTRODUCTION....9
II. HAZARD IDENTIFICATION....12
III. HAZARD CHARACTERIZATION....16
IV. EXPOSURE ASSESSMENT....17
IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)....20
IV. A. 1. UK vCJD prevalence estimated using epidemiological modeling results (Clarke and Ghani 2005) and diagnosed vCJD cases for 2002 and 2003....21
IV. A. 2. UK vCJD Prevalence derived from a Tissue Surveillance study....23
IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2)....24
IV. C. Estimation of the probability that a plasma pool may contain a donation that contains vCJD agent....26
IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood...28
IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in their blood and who may not be deferred by questionnaire screening....34
IV. D. Annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US....37
IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD....39
IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD....40
IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD....41
IV.E. 3. Model results: Estimates of the per vial vCJD infection risk for US manufactured pdFVIII (Module 3)....42
IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation....45
IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII....46
IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients....46
IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients....48

V. RISK CHARACTERIZATION.....49
V.A. THE MODEL.....50
V. B. Model results: Estimated annual potential exposure to vCJD i.v. $ID_{50}$ and potential vCJD risk through human pdFVIII used to treat severe HA.....50
V. C. Model results: Estimated annual potential exposure to i.v. $ID_{50}$ vCJD agent and potential vCJD risk through human pdFVIII used to treat severe von Willebrand disease (vWD).....55
V. D. Sensitivity analysis.....61
V. E. Uncertainty and Data Gaps.....65
V. F. Conclusions.....67

REFERENCES.....69

# TABLE OF TABLES

## EXECUTIVE SUMMARY

Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent .....4
Table I.B. Model Results for Mean Total Population-based Potential vCJD Risk for all Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....5
Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk.....6
Table II.B. Von Willebrand Disease (vWD) patients[a] with Severe Disease: Predicted Total Population-based Potential vCJD Risk.....7

## RISK ASSESSMENT

Table 4.1. FDA model estimation of UK vCJD cases for years 2002 – 2080.....21
Table 4.2. Summary of surveillance testing of tonsil and appendix tissues in the UK.....23
Table 4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups .....26
Table 4.4 Model Results: Annual Number of US plasma donors predicted by model to be potentially infected with vCJD and donate to plasma pools used to manufacture pdFVIII..... 36
Table 4.5 Annual Percentage of US Plasma Pools Potentially containing a vCJD Donation.....38
Table 4.6 Reduction factor (RF) of fractionation procedures.....42
Table 4.7 Annual Predicted per Vial vCJD Infection Risk for US Manufactured pdFVIII from Model.....44
Table 4.8 Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution.....47
Table 4.9. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution..49
Table 5.1A. Model Results for All HA Patients who use a Hypothetical Factor VIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....53
Table 5.1B. Model Results for Total Population-based Exposure and Potential vCJD Risk for All Hemophilia A patients who use a Hypothetical pdFVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....54
Table 5.2A. Results von Willebrand Disease (vWD) patients[1] with Severe Disease: Predicted Potential Annual Exposure to vCJD i.v. $ID_{50}$ and vCJD Risk.....55
Table 5.2B. Von Willebrand Disease (vWD) Patients[1] with Severe Disease: Predicted Total Population-based Exposure to vCJD i.v. $ID_{50}$ and Potential vCJD Risk.....56
Table 5.3A. Range of Predicted Annual Mean Potential per HA Patient vCJD risk for pdFVIII..... 59
Table 5.3B. Range of Total Population-based Exposure and Potential vCJD Risk from Model .....60
Table 5.4. Input Variables included in Importance Analysis .....62

# TABLE OF FIGURES

Figure 1. Exposure Assessment diagram.....19
Fig 2. A. Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using prevalence estimate encompassing the range of values for both high and low prevalence from 0.7 to 700 vCJD cases per million UK population.....63
Fig 2. B. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Tissue Surveillance-based (HIGH) prevalence estimate.....63
Fig 2. C. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Epi Modeling-based (LOW) prevalence estimate. Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.....64

# EXECUTIVE SUMMARY

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the U.K. in 1996 – and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the U.K. Since December 2003, there have also been three reports in the United Kingdom (U.K.) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness.

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the U.K., where the risk is considered greatest, or elsewhere in the world. U.K. authorities have notified physicians in the U.K. and their patients who received plasma derivatives made from plasma from U.K. donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

This document "Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US" quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe disease who have used human plasma-derived Factor VIII (pdFVIII) product manufactured in the US. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may have been exposed to the BSE agent during extended travel or residence in the UK, France or other European countries and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. Risk for these products was estimated for the baseline year of 2002 but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. A few major elements of the model greatly influence vCJD risk. The most influential of these are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the risk estimate is significantly affected by the prevalence

of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe. The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

Patients with hemophilia A (HA) have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a glycoprotein circulating in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or within a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with vWD (Type 3) have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII coagulant activity (anti-hemophilic factor) as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually require treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

**Results from the Model**

An important, yet also highly uncertain parameter in driving the risk assessment results is the estimate used for vCJD prevalence in the UK. The prevalence of vCJD in the UK population was estimated in the model using two different approaches. The first approach to estimating vCJD prevalence in the UK was from a study based on epidemiological modeling that was derived using actual reported vCJD cases in the UK combined with an estimate of future vCJD cases (Clarke and Ghani, 2005). Several factors used in epidemiologic modeling approaches are difficult to quantify and add uncertainty to the final estimated number of future vCJD cases. These factors include: the intensity of human exposure to the BSE agent, incubation period, time of infection, and whether illness will develop in individuals who are not homozygous for methionine at codon 129 of PrP. All cases of vCJD to date have occurred in individuals who are homozygous for methionine at this location. Our calculations, based on the Clarke and Ghani study (2005) and diagnosed cases in 2002 and 2003, yielded a prevalence estimate of approximately 1.8 vCJD cases per million in the UK.

Running the model with this vCJD case prevalence estimate (~1.8 per million) produces an estimate suggesting that, on average, there was a 0.027% likelihood that a plasma pool, which then undergoes manufacturing, will contain at least one donation from an individual whose blood contains the vCJD agent. Therefore, on average, more than 99% of the time the model predicts the product as administered will contain no vCJD agent and this is reflected in the (0 – 0) values for the 5$^{th}$ and 95$^{th}$ percentiles shown for the lower prevalence estimate results in Table I.A. (below).

However, it is possible that the prevalence of vCJD in the UK is higher than that estimated above. This could happen if there are people infected who never develop the disease (but can still spread the infection) or if some individuals take extremely long to become ill. Therefore, a second approach to estimating vCJD infection prevalence was used based on a relatively small tissue surveillance study by Hilton, *et al* (2004), which tested stored tonsil and appendix tissues from the UK for accumulation of abnormal prion protein. It yielded a much higher prevalence estimate of 1 in 4,225 (237 infections per million). This study was not controlled using tissues from a non-BSE exposed population and false positive findings cannot be ruled out. It is also not known whether this staining of appendiceal tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate that may be relevant to transfusion risk and therefore should also be considered. Use of these data as the basis for a vCJD infection prevalence estimate which is then used in the model produces a significantly higher estimate suggesting that, on average, if it were correct, there could be a 2.41% likelihood that a plasma pool, which then undergoes manufacturing, may contain at least one donation from an individual whose blood contains the vCJD agent.

### *Estimated annual potential vCJD risk associated with human pdFVIII used to treat severe Hemophilia A*

Results from the model indicate that it is possible that a donor unknowingly infected with vCJD may have donated plasma used in the manufacture of pdFVIII in the US. Output from the model using the lower UK vCJD prevalence estimate (~1.8 in 1 million) indicated that, on average, there is a 0.027% (95% CI: 0 % - 0 % ) likelihood that a plasma pool may contain at least one donation from an individual with the vCJD agent in their blood. Readers may notice that the 5$^{th}$ and 95$^{th}$ percentile intervals for all of the model outputs are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. This means that at least ninety five percent of the time the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, actual model predictions indicated that, at the lower prevalence, 0.027% of the time the exposure to vCJD may be greater than zero. When the model was run using the higher UK vCJD prevalence estimate (1 in 4,225) to derive an estimate for vCJD prevalence in US plasma donors, the FDA model predicted that, on average, there is an approximately 2.41% (95% CI: 0 % - 10 %) likelihood that a plasma pool will contain at least one donation from an individual with the vCJD agent in their blood. For either set of results, the model assumes that if vCJD agent were present, the amount in a plasma pool would likely be reduced or possibly eliminated by processing steps used during the manufacture of pdFVIII product.

Individuals with HA vary in their degree of FVIII deficiency. For simplicity, the model results and this executive summary specifically address potential vCJD exposure and risk for persons with severe HA. FDA estimates that among the total population of 14,000 HA patients in the United States, approximately 1,800 (Table I.A.) have severe disease and use pdFVIII products. FDA obtained data

on FVIII utilization from the Centers for Disease Control (CDC). The data were generated as part of a collaborative effort between CDC and six states in a study conducted from 1993 –1998. Treatment regimens for HA are administered either as prophylaxis to prevent the occurrence of bleeding episodes or on an episodic basis to control bleeding when it occurs. Additionally, inhibitors may be treated with very high doses of pdFVIII to induce immune tolerance. Assuming these patients are treated with a pdFVIII product that has a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent, Table I.A. displays model outcomes for patients treated using either prophylaxis or episodic treatment, and with respect to their inhibitor status.

**Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent:** *Predicted mean potential per person annual vCJD risk using two different UK vCJD prevalence estimates.*

| | | | | **4 - 6 $\log_{10}$ Reduction** | |
|---|---|---|---|---|---|
| | | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| **Treatment Regimen** | **Inhibitor Status** | **Est. Total Number patients in US** | **Mean quantity FVIII used per person per year (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** |
| ***Prophylaxis*** | No Inhibitor | **578** | **157949 IU[c]** (21242 , 382316 ) | 1 in 4.0 million (0-0)[d] | 1 in 54,000 (0 - 1 in 12,000) |
| | With Inhibitor – No Immune Tolerance | **63** | **190523 IU[c]** (26956 , 447639) | 1 in 4.8 million (0-0)[d] | 1 in 41,000 (0 - 1 in 9,000) |
| | With Inhibitor – With Immune Tolerance | **62** | **558700 IU[c]** ( 33235, 1592943) | 1 in 1.3 million (0-0)[d] | 1 in 15,000 (0 - 1 in 3,700 ) |
| ***Episodic*** | No Inhibitor | **946** | **85270 IU[c]** ( 4633, 244656) | 1 in 9.4 million (0-0)[d] | 1 in 105,000 (0 - 1 in 24,000 ) |
| | With Inhibitor | **151** | **160458 IU[c]** (5314 , 488906 ) | 1 in 8.0 million (0-0)[d] | 1 in 48,000 (0 - 1 in 12,000 ) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[d] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

The risk estimate for the entire severe HA population of 1,800 in the US who use pdFVIII, obtained by summing the total annual exposure and vCJD risk, is shown in Table I.B. Variant CJD risk for US donors with a history of travel to the UK, France or other countries in Europe since 1980 is further adjusted to account for donor age, country, duration and year of travel. Using the lower UK prevalence estimate as a starting point, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 3,077 years of treatment. If the higher vCJD prevalence estimate is used, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 35 years of treatment.

**Table I.B. Model Results for Mean Total Population-based Potential vCJD Risk for all Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent.** Risk estimates were calculated for patients with severe disease, using two different UK vCJD prevalence estimates.

| | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| | **Est. Total Number severe HA patients in US** | **Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c]** | **Mean population –based potential vCJD risk[a] (5th - 95th perc)[b]** | **Mean population –based potential vCJD risk [a] (5th - 95th perc)[b]** |
| ***Mean Total cumulative annual exposure and population risk*** | **1,800** | **243 million IU[c]** | 1 in 3,077 years (0-0)[d] | 1 in 35 years (0 - 1 in 8 ) |

[a] Mean population-based potential annual vCJD risk – the risk of potential vCJD infection for the entire population of 1,800 based on animal model dose-response information.

[b] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[d] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

***Estimated annual potential vCJD risk associated with human pdFVIII used to treat severe von Willebrand disease (vWD)***

Individuals with vWD have varying severities of disease; those with Type 3 disease have the severest form of the disease. This executive summary specifically addresses potential vCJD exposure and risk for persons with severe vWD (Type 3) who are assumed to use larger amounts of pdFVIII product and thus, may be at higher risk. FDA estimates that approximately 250 vWD patients have severe vWD disease in the United States and use human pdFVIII products to control their disease (Table II.A.). Results from the risk assessment model for young vWD patients and adult vWD patients treated with pdFVIII product that is assumed to have a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in Table II.A. Generally results from the model are expressed for patients with vWD for two groups, either Prophylaxis or Episodic treatment. FDA obtained data on FVIII utilization from the Centers for Disease Control (CDC). The data were generated as part of a collaborative effort between CDC and six states; the study was conducted from 1993 –1998. Annual usage of product by vWD patients was estimated based on an assumption that this patient class largely uses Humate-P®. Totaling the model results for the lower prevalence estimate of ~1.8 per million reveals that the 250 severe vWD patients in the US (Table II.B.) are predicted to have an average potential vCJD infection risk for the population of 1 infection in 28,450 years. At the higher prevalence estimate, the average potential vCJD infection risk for this population is 1 infection in 405 years

**Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk:**

- Assuming a reduction from manufacturing of 4-6 log $_{10}$ , and
- Two different UK vCJD prevalence estimates.

| YOUNG vWD (≤ 15 yrs of age) | | | | |
|---|---|---|---|---|
| | | | **4 - 6 $Log_{10}$ Reduction** | |
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| | **Est. Total Number patients in US** | **Mean quantity product used per person per year** | **Mean vCJD risk per person per year[b] (5th - 95th perc)[c]** | **Mean vCJD risk per person per year[b] (5th - 95th perc)[c]** |
| ***Prophylaxis*** | **39** | **165,713 IU[d]** (9876, 454306) | 1 in 4.7 million (0-0)[e] | 1 in 52,000 (0 - 1 in 13,000) |
| ***Episodic*** | **60** | **11,045 IU[d]** (1025, 34352) | 1 in 48 million (0-0)[e] | 1 in 971,000 (0 - 1 in 293,000) |
| **ADULT vWD ( > 15 yrs of age)** | | | | |

| | | | | |
|---|---|---|---|---|
| *Prophylaxis* | 73 | 186,880 IU[d] (16910, 539877) | 1 in 4.1 million (0-0)[e] | 1 in 46,300 (0 - 1 in 11,000) |
| *Episodic* | 78 | 86,923 IU[d] (2182, 240338) | 1 in 10 million (0-0)[e] | 1 in 1 million (0 - 1 in 24,000) |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

**Table II.B. Von Willebrand Disease (vWD) patients[a] with Severe Disease: Predicted Total Population-based Potential vCJD Risk:**

- *Assuming a reduction from manufacturing of 4-6 log $_{10}$, and*
- *Two different UK vCJD prevalence estimates.*

| | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| | **Est. Total Number severe vWD patients in US** | **Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c]** | **Mean[b] population –based Potential vCJD risk (5th - 95th perc)[c]** | **Mean[b] population –based Potential vCJD risk (5th - 95th perc)[c]** |
| ***Mean total annual exposure and population risk*** | **250** | **29.9 million IU[d]** (3013, 311745) | **1 in 28,450 years** (0 - 0)[e] | **1 in 405 years** (0 - 1 in 76) |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or sodic treatment with Humate P only and no record of inhibitor.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

## Conclusions

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Although donor deferral criteria in place

since 1999 have reduced the risk of donation by exposed persons, some are not deferred and potentially may donate plasma that contains the vCJD agent. However, the model suggests that the likelihood of a vCJD contaminated plasma pool is low.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the model predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from1 in 15,000 for the higher vCJD prevalence estimate and high product usage to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. Due to the wide range of methods used for currently available clearance studies, the results themselves, and gaps in information, it is not possible at this time to determine with any certainty if a specific product may be less or more safe than another.

Although results of the model suggest exposure to vCJD agent is possible, and there is a potential risk of infection that is likely to be very low, it is not possible for the model to provide a *precise* estimate of the vCJD risk in general, or of the actual risk to individual patients. Although the actual risk is highly uncertain, the risk assessment model indicates that the most important factors affecting risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, and the vCJD prevalence in the UK donor population.

*In considering the results of the risk assessment it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products, including pdFVIII. This includes patients receiving large amounts of plasma-derived products manufactured from UK plasma donations over a long period of time.* This observation also suggests that the actual risk of vCJD infection from pdFVIII is likely to be very low. The absence of cases does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

# RISK ASSESSMENT

## I. INTRODUCTION

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the UK in 1996 – and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the UK. Since December 2003, there have also been three reports in the United Kingdom (UK) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness.

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the UK, where the risk is considered greatest, or elsewhere in the world. UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

Because only 3 cases of BSE (2 that originated in the US, 1 in Canada) have been reported in the US, the US vCJD risk from domestic beef is thought to be very low. However, some US residents (including blood and plasma donors) traveled to the UK, France and other countries in Europe since 1980 and may have been exposed to the BSE agent, and some of these donors may unknowingly be infected with vCJD. The UK had the largest epidemic of BSE among its cattle population and the largest human epidemic of vCJD, which as of August, 2006, reported 162 cases. The UK instituted strong food chain control measures to prevent the entry of high risk cattle tissues into its food supply in 1996; so risk after that time likely decreased considerably. France is considered to rank second in the world for risk for vCJD at this time, albeit at a much lower level than the UK, but higher than many other countries in Europe. As of August 2006 France has reported 20 cases of vCJD. Current US blood and plasma donation policies defer donors with a history of travel or residence to: the UK for a period of three months or longer (1980 – 1996); France, for a period of five years or longer (1980 - present); and other countries in Europe (blood donation only) for 5 years or longer (1980 – present). The CJD geographic donor deferral policy likely removes most of the vCJD risk; however, there may be residual risk in the US donor population for persons who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process.

FDA initiated a draft risk assessment of the potential vCJD risk for US manufactured pdFVIII in late 2004. A preliminary draft concept risk assessment model assessing the potential vCJD risks for US manufactured pdFVIII was presented at the February 8, 2005 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for review and comment. The committee largely agreed with the proposed approach. On October 31, 2005, FDA sought advice and

discussion on several risk assessment model inputs to be used in a risk assessment for US manufactured pdFVIII and potential vCJD risks. FDA has incorporated staff, peer reviewer comments, and technical advice provided by the TSEAC at the February 8, 2005 and October 31, 2005 meetings to develop this document "Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US".

This document quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe (Type 3) disease who have used human pdFVIII product manufactured in the US. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may still have been exposed to the BSE agent during extended travel or residence in the UK, France or countries of Europe and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

## Scope of the risk assessment

The scope of this FDA risk assessment evaluates the annual potential exposure to the vCJD agent and risk of vCJD infection through human plasma-derived Factor VIII (pdFVIII) product manufactured in the US. Risk for these products was estimated for the baseline year of 2002 but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. The FDA risk assessment specifically addresses pdFVIII used to treat patients with severe HA and severe vWD.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. A few major elements of the model greatly influence vCJD risk. The most influential are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the prevalence of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe, has a significant effect on the risk estimate.

The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to

provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

## Background

*Hemophilia and Factor VIII*

Patients with HA have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a protein in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with vWD have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually necessitate treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

FVIII from human plasma is manufactured in a number of different ways. FVIII manufactured from human plasma is purified by fractionation of the protein from large plasma pools containing thousands of donations of plasma. Because thousands of donations are used to assemble the plasma pools used in the manufacturing of pdFVIII, there is a possibility that a donation from a vCJD infected individual may be present in a large plasma pool used to manufacture pdFVIII. In turn, that may lead to exposure of product recipients to the vCJD agent and a risk of infection. Relatively recent advances in pdFVIII production technology, ----------, have likely reduced potential exposure to the vCJD agent. However, further evaluation is necessary to more precisely determine the levels of vCJD clearance afforded by the manufacturing processes for each human pdFVIII product.

There are two approaches for the clinical treatment and control of HA using pdFVIII: (1) episodic treatment and (2) prophylaxis. Episodic treatment involves the administration of FVIII in response to bleeding episodes resulting from trauma or during and after surgery. Prophylaxis treatment requires administration of clotting factor concentrates on a regularly scheduled basis necessary to maintain a minimal level of FVIII (common acceptable trough level is 2-5% of base line level) to prevent bleeding episodes. In view of the demonstrated benefits of prophylaxis, the Medical and Scientific Advisory Council (MASAC) recommends that prophylaxis starting at an early age be considered as an optimal therapy for individuals with severe HA (MASAC 2001). Prophylaxis treatment requires

higher doses of FVIII than episodic treatment (Linden, Kolakoski *et al* 2003; Globe, Curtis *et al* 2004) and thus presents a potentially higher risk of vCJD to the patients than episodic treatment when human pdFVIII is used. Also, some HA patients develop antibodies to FVIII, called inhibitors, that limit the effectiveness of FVIII used in treatment. Inhibitors can develop with the use of either recombinant FVIII or pdFVIII products. In some cases the development of inhibitors is treated with immune tolerance therapy in which large doses of one million or more units of pdFVIII may be administered. Because of the large doses of pdFVIII used, immune tolerance therapy can pose a potential risk for vCJD exposure if vCJD agent were present in the pdFVIII product. As a simplifying assumption in the model we assumed that in a given year a patient received either exclusively prophylaxis treatment or episodic treatment, but not both.

### Risk Assessment Framework

This risk assessment generally follows the four step paradigm described by the National Research Council (NRC, 1983) and consists of: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. The hazard identification portion of the risk assessment provides an in-depth overview and analysis of all data and information sources to establish a causal relationship between the hazard and adverse effects on humans. The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the probability of adverse consequence(s) such as infection, illness, etc., expected at a given dose at the individual, subpopulation, or population level. Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. Risk Characterization integrates the information from the hazard identification, hazard characterization and exposure assessment sections to characterize the probability and consequences of risk for individuals and populations.

## II. HAZARD IDENTIFICATION

The hazard identification portion of the risk assessment provides an in-depth overview and analysis of information from laboratory studies, epidemiological studies, the scientific literature, government reports and other credible or peer-reviewed sources of data that establish a causal relationship between the hazard and adverse effects on humans. In this risk assessment, the vCJD agent is the hazard, and potential exposure can occur in individuals who use plasma-derived products that may have been manufactured from plasma that may have contained a donation(s) from a vCJD-infected individual. To date, there is no epidemiological evidence suggesting that vCJD has been transmitted by use of plasma derivative products. However, the probable transmission of vCJD to three recipients of red blood cell products donated by donors later diagnosed with vCJD in the UK raises the possibility of vCJD transmission via plasma-derived products.

Human vCJD was first reported in the United Kingdom in 1996 (Will *et al* 1996). As of August 2006 over 195 cases, 162 of them in the UK, have been reported worldwide. Both vCJD and BSE belong to a class of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). There is strong evidence and general agreement that vCJD results from infection of humans

most probably via dietary exposure to bovine spongiform encephalopathy (BSE)-contaminated beef (Knight 2004). The leading theory is that the transmissible infectious agent is a prion, or proteinaceous infectious agent, that is an altered but pathogenic form of the PrP protein that is normally present in cells. The altered PrP, herein referred to as $PrP^{TSE}$, consistent with terminology recommended by the World Health Organization, is highly stable and resistant to degradation by high heat and chemical treatments commonly used to denature infectious agents in the manufacture of plasma derivatives. The incubation period for TSEs is long. The mean incubation period of BSE in cattle is approximately 4.5 years. In humans, vCJD acquired through dietary exposure is thought to incubate approximately 10 –12 years or longer, and individuals become symptomatic only in the last few months of the disease, making early detection very difficult. Confirmation of vCJD requires postmortem examination of brain tissue to confirm diagnosis, but prion protein has been detected in tonsil and appendix tissue of asymptomatic individuals as long as two years prior to the onset of symptoms. There are currently no validated tests available to detect the disease in its early stages of infection or to detect the presence of TSE agents in blood.

**Transmission of TSEs through transfusion of blood products in animal models**

Transmission of different TSE agents through the transfusion of blood or blood products has been demonstrated in animal models on multiple occasions. At least four studies reported transmission via blood transfusion in the same animal species: sheep experimentally infected with BSE (Houston *et al* 2000), sheep naturally infected with scrapie (Hunter *et al* 2002), hamsters with scrapie (Rohwer 2004), and mice with a human TSE (Brown *et al* 1999).

Brown, Rohwer, Taylor (Taylor *et al* 2000) and others have attempted to estimate the amounts of intracerebral (i.c.) infectivity present in blood, which generally fell between 2 and 20 i.c. $ID_{50}$/ml. A recent study of scrapie-infected hamsters concluded that approximately 58% of the infectivity present in whole blood was associated with plasma (Gregori *et al* 2004). The model uses this more conservative estimate in the published literature and assumes that 58% of infectivity is associated with plasma.

**Transfusion transmission of vCJD in the United Kingdom**

In December 2003 the UK government announced that vCJD had likely been transmitted to a 69 year-old patient via blood transfusion. The patient had received non-leukoreduced red blood cells in 1996 from a donor who died three years later of vCJD. This first report was followed by the announcement in July 2004 of another probable case of transfusion-transmitted vCJD. The patient died of a ruptured aortic aneurysm without clinical evidence of vCJD, but postmortem testing detected $PrP^{TSE}$ in spleen tissue and cervical lymph node. In February 2006 a third case of probable transfusion transmitted vCJD was reported in the UK in a 31 year-old male; the patient had received a transfusion eight years earlier from a donor who died of vCJD 20 months after donation. None of the donors were known to have had vCJD at the time of donation.

It is possible that dietary exposure may have been responsible for some or all of the three cases that were reported after red blood cell transfusions; however, the probabilities for occurrence of either a single, or, particularly, two or three such events are small. As Llewelyn *et al* (2004) pointed out in their publication discussing the first presumed transfusion-transmitted case "the age of the patient

was well beyond that of most vCJD cases, and the chance of observing a case of vCJD in a recipient in the absence of transfusion transmitted infection is about 1 in 15,000 to 1 in 30,000." The combined probability that the first two transfusion cases, identified in two elderly patients in a small cohort of transfusion recipients—in an age group underrepresented among vCJD cases—both acquired infection from food is remote.

The presumptive transmission of vCJD via red blood cell transfusion in the UK raises the possibility that plasma derivatives may pose a risk. The UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These plasma derivative products included coagulation factors, as well as antithrombin III, and intravenous immune globulins. The derivatives of concern were manufactured from plasma of UK donors between 1980 and late in 1999, when--consistent with a decision announced in 1998—UK manufacturers stopped using UK plasma. The last expiry date for any of the UK products was in 2001. To date, no cases of vCJD have been reported in any recipients of plasma derivatives, either in the UK, where the risk is considered greatest, or elsewhere, including in patients who have received human plasma-derived coagulation products from implicated lots (e.g., lots manufactured from a pool containing plasma from a donor subsequently diagnosed with vCJD) made in the UK.

**The vCJD risk for travelers with history of extended travel or residence in the UK, France, and other countries in Europe and reduction of risk via donor deferral**

Public health control measures, such as surveillance, culling of sick animals, or banning specified risk materials, and others have been instituted relatively recently in many European countries, particularly in those with indigenous cases of confirmed BSE, in order to prevent potentially BSE-infected tissues from entering the human food supply. Since 1996, the UK has instituted some of the most stringent of these control measures, including a program that excludes all animals >30 months of age and prevents high risk tissue from slaughtered animals from entering the human food and animal feed supplies. In June 2000, the European Union Commission on Food Safety and Animal Welfare strengthened the European Union's BSE control measures by requiring all member states to remove specified risk materials from animal feed and human food chains; as of October 1, 2000 such bans had already been instituted in most member states.

Travelers to and residents of the UK, France and other countries in Europe during the period of BSE pandemic concern are possibly at increased risk of vCJD. However, the risk can not be determined precisely due to factors such as the great uncertainty about incubation period of the disease, the sensitivities of each country's surveillance for BSE and vCJD, the compliance with and effectiveness of public health measures instituted in each country to prevent BSE contamination of human food, and the cattle products from one country that are distributed and consumed elsewhere.

In the UK, the current risk of acquiring vCJD from eating beef and beef products appears to be extremely small, perhaps about 1 case per 10 billion servings (CDC, 2005). In the other countries of the world, this current risk, if it exists at all, would not likely be any higher than that in the UK if BSE-related. The implementation of animal and public health control measures has caused the prevalence of BSE to decline. The US blood donor deferral criteria currently in effect focus on the

time (cumulatively 3 months or more) that a person lived in the UK from 1980 through 1996, whereas for the rest of Europe the criteria focus on the time (cumulatively 5 years or more) that a person lived in these countries from 1980 through the present. This deferral policy is expected to reduce the risk of vCJD transmission via blood and plasma donations from potential infected donors.

**Two cases of vCJD in US residents with history of residence in the UK**

In 2002, the first case of vCJD was reported in the United States in a 22-year-old woman living in Florida. She is believed to have become infected with vCJD during her residence in the UK. The patient was born in Great Britain in 1979 and immigrated to the United States in 1992. In early November 2001, the patient was evaluated for depression and memory loss. In late January 2002, the patient was transported to the United Kingdom where her condition worsened. The diagnosis of vCJD was confirmed by western blot and immunohistochemical analysis. The patient died in June 2004, approximately 32 months after illness onset (Belay *et al* 2005).

A second case of vCJD was diagnosed by the UK National Creutzfeldt-Jakob Disease Surveillance Unit in November 2005 in a 30-year old man who resided in Texas during the period 2001-2005 (CDC 2006). The onset of symptoms occurred in early 2005 while the man was in Texas. He returned to the UK and died of the disease in early 2006. A postmortem examination confirmed the diagnosis of vCJD.

**Surveillance studies to detect CJD and vCJD in patients with hemophilia**

**Studies in the United States**
Because of the large number of blood products used, persons with hemophilia might be expected to be at risk of developing transfusion-related vCJD or classical Creutzfeldt-Jakob disease (CJD). However, a study conducted by the US Centers for Disease Control (CDC) (Evatt *et al* 1998) examined the brains of 24 decedents with a history of bleeding disorders and dementia and found no evidence of CJD in any of the cases.

Another study conducted by the CDC and the Hemophilia Treatment Center identified no cases of clinical diagnosis of CJD among over 12,000 HA patients who have been assessed since 1996. (Evatt *et al* 1998)

**Studies in the United Kingdom**
A study in the UK (reference: Lee *et al* 1998) conducted post mortem histological examination of the brains of 33 hemophilia patients who were treated with coagulant factor concentrates spanning the years from 1962 – 1995 and observed no evidence of vCJD.

In summary, the experimental and epidemiological evidence indicates the risk of blood transmission of vCJD is no longer theoretical but a real possibility. To date, there is no evidence suggesting that either human CJD or vCJD have been transmitted by use of plasma derivative products. However, transmission of vCJD via red blood cell tranfusions has likely occurred (Llewelyn *et al* 2004). Because the vCJD agent may be associated with plasma, it is plausible that plasma derivatives potentially pose a risk of transmitting the disease.

# III. HAZARD CHARACTERIZATION

The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the adverse consequence(s) such as infection, illness, etc., at the individual, subpopulation, or population level. Determining dose-response relationships can be difficult to accomplish because data are limited, especially exposure and outcome data for humans. Other factors such as characteristics of the hazard (e.g. strain, chemical make-up, etc.), route of introduction, genetics of exposed individuals, influence the dose-response relationship but are often difficult to characterize. Often in lieu of human data, animal data are used and appropriately extrapolated as best as is possible to estimate the dose-response relationship for humans.

Another challenge is estimating the probability of infection when the exposure to TSEs is small and/or occurs repeatedly over a period of time. It is unknown whether for TSE diseases there is a minimal amount of the agent (presumably the prion protein $PrP^{TSE}$) or threshold that is needed to initiate infection in an individual. This phenomenon is seen with many other pathogens such as viruses or bacteria, for which infection requires exposure to at least one, and often more, units of the infectious agent. Furthermore, it is not known whether the effects of small multiple exposures over a period of time are cumulative and may result in the possibility of infection and disease equivalent to a single, larger exposure (e.g., via intracerebral injection in laboratory animals). Some risk assessments have made assumptions concerning the exposure and dose for TSE agent that leads to infection. For instance, the Det Norske Veritas (Feb 2003) blood products risk assessment assumes that exposure to infectivity, quantified in $ID_{50}$ units, is cumulative over the period of one year. Based on advice from the TSEAC (2005), and consistent with suggestive data from studies of TSE agents in animal models (Diringer *et al* 1998, Jacquemot, *et al* 2005), FDA also assumes that exposure to vCJD $ID_{50}$ is cumulative over a one year period. The $ID_{50}$ is the common metric used to quantify the infectivity of TSEs. One $ID_{50}$ is defined as the amount of infectious material or tissue that is necessary to initiate infection in 50% of the treated population. The route of exposure to TSE infectious material influences the efficiency of transmission of the disease. Based on advice provided to FDA by the TSEAC (October 31, 2005) the model assumes that transmission via the intravenous (i.v.) route is between 1 and 10 times less efficient than the transmission via the intracranial (i.c.) route.

In estimating the dose-response relationship for TSEs one could use a strict interpretation of the $ID_{50}$ and assume a linear relationship between exposure and infection. In the pdFVIII model FDA assumed there was a linear relationship between the exposure dose of vCJD agent and the probability of infection. The $ID_{50}$ relationship used in the model was based on infectious TSE units estimated from rodent model studies (Brown 1998, 1999; Rowher 2004). We further assumed there was no threshold or minimum dose necessary to initiate infection, that is, exposure to even low quantities of vCJD agent has a probability of initiating infection in an individual, albeit the probability of infection would likely be low at low levels of exposure. The model further assumes that in such a case exposure to 1 $ID_{50}$ would suggest a 50% probability of infection, exposure to 0.1 $ID_{50}$ would suggest a 5% probability of infection, and so on. However, given the lack of information and high degree of uncertainty on the dose-response relationship because of the limited data available for TSE agents, it is plausible that low level exposures, even on a chronic basis, may not attain a threshold or minimum quantity of agent necessary to initiate infection in humans. Again, FDA makes a conservative

assumption that low-level exposure(s) over the period of one year to any quantity of vCJD agent could potentially lead to infection and that there is not a minimum dose necessary to initiate infection.

There are considerable uncertainties in determining the correct form for the vCJD-human dose-response model. For instance, the nature of the dose-response line, its slope, or whether it is more accurately described using a dose-response curve is uncertain because animal data are so limited and human data are not available. The FDA risk assessment estimates the potential individual risk of infection and assumes that a linear interpretation of the rodent model accurately reflects the pathology and progression of vCJD infection and disease in humans, but it may not. Furthermore, exposure to the vCJD agent may not necessarily lead to infection, and vCJD infection may not necessarily produce symptomatic vCJD disease or illness in an individual or population.

# IV. EXPOSURE ASSESSMENT

Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. This exposure assessment specifically addresses the probability of exposure and, if present, the quantity of vCJD agent that may potentially be present in plasma-derived FVIII products manufactured in the United States. The administration of pdFVIII and, thus, the route of exposure, is intravenous.

Plasma pools consisting of 6,000 or more donations collected from US plasma donors are used as the starting material from which a number of plasma-derived products are purified, including pdFVIII, which is addressed in this assessment. Because of the relatively large number of donations per plasma pool, there is a small probability that even in the United States some of the pools may contain a donation from a donor who may unknowingly be infected with vCJD, but who does not meet criteria for donor deferral, or who meets those criteria but fails to be deferred due to the limitations of the screening process.

**Overview of Model for pdFVIII**

**Module 1 – Estimation of the prevalence of vCJD in the UK.** Variant CJD prevalence in the UK was used in our model as the basis for estimating vCJD prevalence in US plasma donors. The model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and may have had dietary exposure to the BSE agent during their stay.

Two different data sources were used to estimate UK vCJD prevalence.

- An epidemiological modeling-based approach estimates a UK vCJD case prevalence of approximately ~1.8 cases per million population (Clarke and Ghani 2005).
- A tissue surveillance-based estimate for UK vCJD infection prevalence was generated using data from Hilton *et al* (2004) and yielded a mean estimate of 1 case per 4,225 – but was further adjusted to account for age of patients surveyed.

**Module 2 –vCJD Prevalence in US Plasma Donors and Pools.** This module estimates the number of US plasma donors that may potentially be infected with vCJD and the percentage/number of pools containing donations with vCJD agent. This module uses survey data to determine US plasma donors potentially at risk for vCJD, including those with a history of:

- Dietary exposure to BSE-contaminated beef during long term travel or residence in the UK (1980-1996), France and other countries in Europe (since 1980),
- Military service - posted on or residing near military facilities in Europe; and
- Transfusion with blood collected in Europe, or Euroblood.

US plasma donors potentially at risk for vCJD were further characterized by:

- Country of travel or residence,
- Specific duration of travel or residence, year of travel or residence,
- Age of donor, rate and frequency of plasma donation,
- Number of donations per pool, and type of plasma pool (source or recovered), and
- Effectiveness of donor deferral policies.

**Module 3 - pdFVIII Manufacturing and Processing.** This portion of the model calculated the likelihood and number of plasma pools potentially containing vCJD agent and the quantity of agent per plasma pool and pdFVIII vial based on:

- The probability of and predicted quantity of infectivity (i.v. $ID_{50}$) present per donation and pool
- Reduction in the quantity of potential vCJD agent during manufacture, and
- Total yield or quantity of pdFVIII produced from the plasma pool.

**Module 4 - Utilization of pdFVIII by Hemophilia A patients.** The potential exposure of an individual HA patient to the vCJD agent through use of pdFVIII was estimated in the model based on:

- the total quantity of pdFVIII used per year, and
- the estimated potential quantity of vCJD agent predicted in the pdFVIII product.

The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen and was estimated using data from a Centers for Disease Control (CDC) sponsored study by 6 states by HA patients from 1993-1998

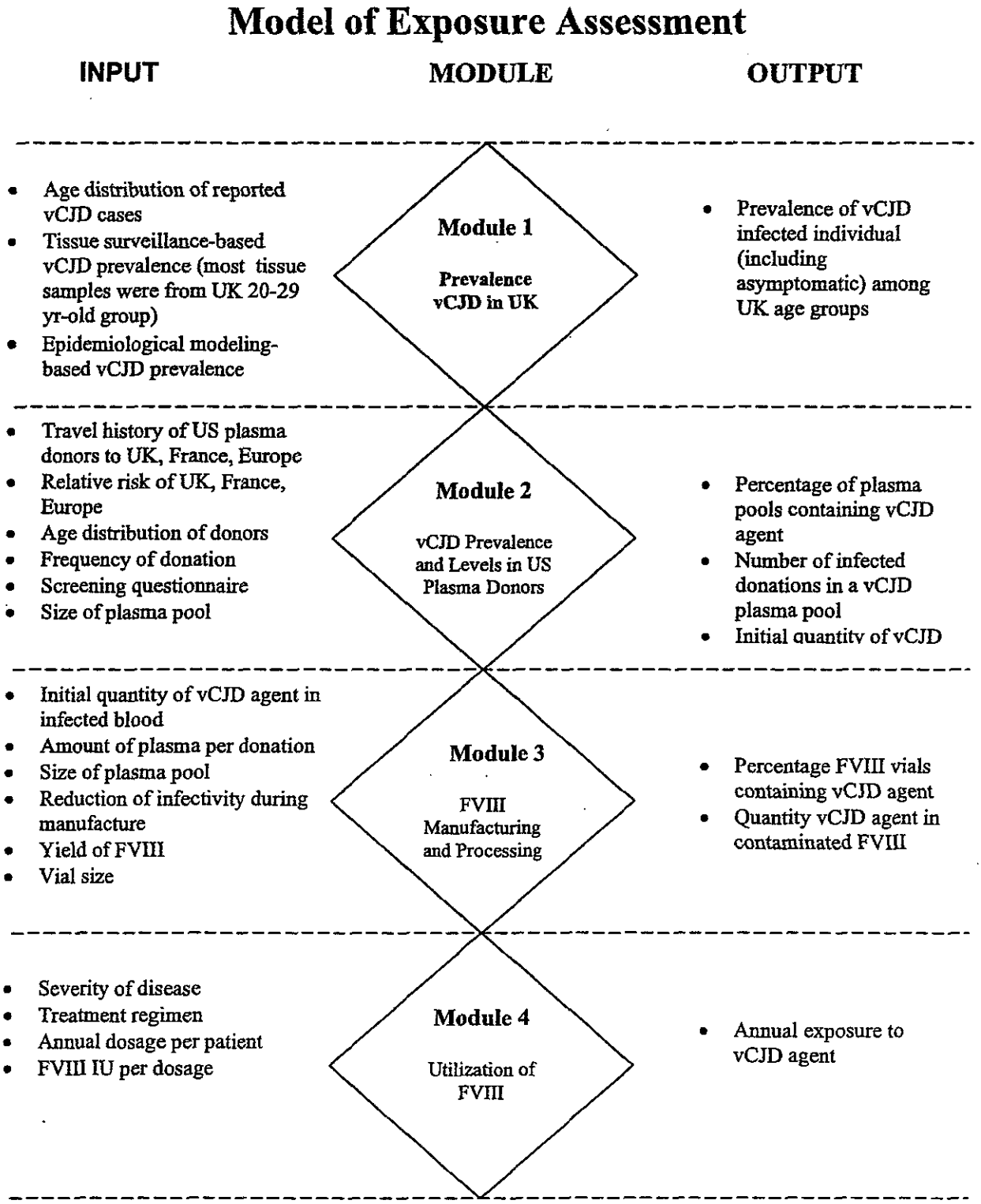
This risk assessment provides outputs that estimate annual exposure for several patient subpopulations with

**Severe HA** disease for persons in the following clinical treatment groups:

- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

**vWD** for adult ($\geq$15 yrs of age) and young ($\leq$15 yrs of age) persons, including those in either clinical treatment group: Prophylaxis or Episodic.

Figure 1 Exposure assessment diagram



## IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

The potential prevalence of vCJD in the UK was and continues to be dynamic and changes throughout time as people are exposed to the BSE agent, infected with vCJD, develop the disease and eventually die. Variant CJD exposure and infections in the UK population likely occurred in proportion to the UK BSE epidemic which peaked in 1992. The first human vCJD cases were referred to UK public health authorities in 1994. To date, the number of cases per year in the UK reached a maximum of 28 in the year 2000, and since then has been declining annually with a total of 5 deaths in year 2005.

The FDA model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and who may have had dietary exposure to the BSE agent during their stay. The potential vCJD prevalence in US plasma donors with a history of travel to BSE countries since 1980 was estimated based on the UK vCJD prevalence. For US donors the UK vCJD prevalence was adjusted based on the proportion of time spent in the UK, the year of travel and age of the donor. Calculation of the potential vCJD risk for donors who traveled to France was estimated relative to the UK risk (or the relative risk), based on the amount of UK beef imports, the number of domestically acquired vCJD cases, and other factors. The relative risk for vCJD for France was assumed to be 0.05 times that of the UK risk (or 0.05 times the UK vCJD prevalence). Applying similar criteria for other countries in Europe their relative risk was assumed to be 0.015 times that of the UK. Risk was calculated in the model for donors by multiplying the UK vCJD prevalence by either 0.05 for travel to France or 0.015 for travel to other European countries and further adjusting the prevalence to account for factors such as the proportion of time spent, the year of travel and age of the donor.

The prevalence of vCJD in the UK is difficult to estimate because of the long incubation period of the disease and a lack of a validated test that can detect infection in its asymptomatic stages. The prevalence of asymptomatic vCJD infections in the UK was estimated in the FDA model using two different approaches based on two different data sources:

- **An epidemiological modeling-based approach that combined information from a study by Clarke and Ghani (2005) and diagnosed vCJD cases for 2002 and 2003 was used to estimate a UK vCJD case prevalence of approximately ~ 1.8 cases per million population.** There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. Several factors used in epidemiologic modeling approaches are difficult to quantify and add uncertainty to the final estimated number of future vCJD cases. These factors include: the intensity of human exposure to the BSE agent, incubation period, time of infection, and whether illness will develop in individuals who are not homozygous for methionine at codon 129 of PrP. All cases of vCJD to date have occurred in individuals who are homozygous for methionine at this location. A more detailed description of the derivation of the epidemiological modeling-based estimate and further discussion of the limitations of the approach can be found in section IV. A. 1. below.

- **A tissue surveillance-based estimate for UK vCJD prevalence was generated using the results of a UK study that tested stored tonsil and appendix tissues collected from patients in the 1990s for the accumulation of prion agent (Hilton *et al* 2004).** The study yielded a much higher estimate of 1 in 4,225 (237 infections per million). However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be

considered. A total of 3 positive appendix tissues were identified among 12,676 tissue samples tested, yielding a mean UK vCJD prevalence estimate of 1 case per 4,225. This prevalence estimate was further adjusted to account for the age of the patients surveyed (mostly 20 – 29 year olds) to arrive at a total population-based estimate of UK vCJD prevalence.
This study was not controlled using tissues from a non-BSE exposed population, and false positive interpretations of the findings cannot be ruled out. It is also not known whether this staining of appendiceal tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be considered. A more detailed description of the derivation of the tissue surveillance-based estimate and further discussion of the limitations of the tissue surveillance study can be found in section IV. A. 2. below.

Two spreadsheet models were developed for the FDA risk assessment – one for each of the two prevalence estimates – but otherwise the models were identical in all other ways. We describe the surveillance variables and assumptions in the sections immediately below.

## IV. A. 1. UK vCJD prevalence estimated using epidemiological modeling results (Clarke and Ghani 2005) and diagnosed vCJD cases for 2002 and 2003

The first approach used to estimate UK vCJD prevalence in the FDA model relied largely on epidemiological modeling results (Clarke and Ghani 2005) that estimated future 70 vCJD cases in the UK for the years 2004 – 2080. Since the FDA model estimates the baseline vCJD infection risk for pdFVIII product used in the year 2002, we assumed the potential risk for US donors should be calculated based on a UK vCJD prevalence that included all vCJD cases and potentially incubating vCJD infections in the year 2002. Therefore to estimate the number of cases and future vCJD infections in the UK for the years 2002 – 2080 we added the 32 known diagnosed cases in years 2002 and 2003 and the estimated future 70 vCJD cases (Clarke and Ghani 2005). We assumed that the 70 future cases predicted by Clarke and Ghani (2005) would be incubating vCJD infection in 2002. Therefore, the FDA model estimated an average of 102 cases and incubating vCJD infections for the year 2002 and assumed a 95% confidence interval of 42 – 222 cases. The results of the input information and calculations for the number of vCJD cases in the UK in 2002 are summarized in **Table 4.1**. Assuming the population of the UK in 1997 is approximately 58 million, the prevalence of vCJD (United Kingdom Office for National Statistics, 1997) would be a mean of approximately 1.8 vCJD infections per million population (102 potential vCJD cases / 58 million).

**Table 4.1. FDA model estimation of UK vCJD cases for years 2002 – 2080.**

| | **Diagnosed vCJD cases in the UK** (Health Protection Agency, 2006) | | | **Estimation of future UK vCJD cases** (Clark and Ghani 2005) | **FDA model: Estimation of UK vCJD cases for years 2002 - 2080** |
|---|---|---|---|---|---|
| **Year(s)** | **2002** | **2003** | Total | **2004 - 2080** | **2002 - 2080** |
| **Number of vCJD cases** | 16 | 16 | 32 | 70 (10 – 190) | 102 (42 – 222) |

There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. Many of the published models of future vCJD cases or vCJD incidence in the UK, including Clarke and Ghani (2005) and Cooper and Bird (2003), use simplifying assumptions in generating their predictions. Although these simplifying assumptions are a necessary part of vCJD case estimation efforts, they contribute considerable uncertainty to the final case estimates. Generally, the types of assumptions used to estimate vCJD cases fall into four general areas. First, the models must estimate the number of clinical and pre-clinical BSE-infected cattle slaughtered in the UK to estimate the intensity of human exposure to the BSE agent. Second, they assume a level of effectiveness of the 1989 Specified Ban on Offals which was assumed to reduce the quantity of infectious BSE agent in the food supply, thereby reducing human exposure in the UK. Third, the models generate an appropriate mathematical representation (or statistical distribution) for the incubation period, which is represented by many using a unimodal statistical distribution. There may be constraints on the incubation period used in the model (e.g., the vCJD incubation period of all individuals in the population would not exceed 40 years, etc.). Fourth, many of the modeling approaches incorporate age-specific dependencies that influence exposure, susceptibility to the disease, and incubation period. Depending on the assumptions used, estimates of future cases of vCJD have varied considerably. Past estimates of vCJD cases from epidemiological models predicted from 250 to 440 future cases under certain assumptions (d'Aignaux et al 2001). As actual reported vCJD cases peaked in 2000 and have since been declining, predicted estimates of future cases have decreased (Boelle et al 2003, Clarke and Ghani 2005, Cooper and Bird, 2003).

There are additional uncertainties in predicting future vCJD cases that might arise from individuals with different genetic backgrounds and susceptibilities in the UK population. To date, all known cases of vCJD have occurred in individuals that were methionine homozygous (MM genotype) at codon 129 of the prion protein gene (PNRP). Recent research has identified two individuals who were valine homozygous (VV genotype, also called non-MM genotype) at PRNP codon 129 (Ironside *et al* 2006) among the three prion protein positive samples identified by Hilton *et al* (2004). Clarke and Ghani (2005) did incorporate the possibility of wider genetic susceptibilities in some of their estimates of future vCJD cases. However, because no cases of clinical vCJD have been identified in individuals with non-MM genotype, it is uncertain whether these individuals will in fact develop or transmit clinical disease. Therefore, any estimation of the incubation period for potential cases with the non-MM genotype would rely heavily on assumptions, which adds considerable uncertainty to any estimate of the size or number of cases in a possible secondary wave of vCJD cases that might occur in non-MM individuals.

## IV. A. 2. UK vCJD Prevalence derived from a Tissue Surveillance study

We used a second approach for estimating UK vCJD prevalence drawing on results from a tissue surveillance study that tested lymphoreticular tissue samples (tonsils and appendices) for prion protein accumulation. The study was a retrospective survey of stored tonsil and appendix tissues surgically removed from UK patients in 1995 and subsequent years. The authors identified appendix samples from 3 patients as positive for lymphoreticular accumulation of prion protein out of a total of 12,674 patient samples tested (Hilton *et al* 2004). No tonsil biopsies showed such findings. The significance of the detection of prion protein in the appendix is not certain, and it is not known whether this test is a reliable marker for either vCJD pre-clinical infection or the ultimate development of disease. Nor is it

known whether or not such detection is a marker for an individual's potential capability to transmit infection through blood donation. Results from the tissue surveillance study are summarized in **Table 4.2.** Assuming the sensitivity and specificity of the testing method is 100%, this translates roughly to a vCJD prevalence of of 237 cases per million (95% CI: 49 – 692 cases per million) for all age groups. The authors (Hilton et al 2005) indicated that approximately 60% of the samples tested (from 7,600 patients) came from patients 20-29 years of age. Among the 20-29 year old group we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million.

**Table 4.2. Summary of surveillance testing of tonsil and appendix tissues in the UK.**

| Reference | Ages of population examined | Years tissue taken | Number of positives | Total samples examined | Rate per million (95% CI) |
|---|---|---|---|---|---|
| Hilton DA, et al. 2004 | 10 – 60+ yrs (60% of patients were 20-29 yrs) | 1995 - 1999 | 3 Appendices | 14,964 Appendices 1,739 Tonsils 4,029 excluded | 237/million (49–692 per million) |

There are some possible limitations of using the Hilton *et al* tissue surveillance study in estimating vCJD prevalence. In their tissue survey, Hilton *et al* stressed that there were uncertainties and suggested caution in attempting a prevalence estimate for infection or a prediction of future vCJD cases in the UK based on detection by immunohistochemical staining of lymphoreticular accumulation of prion protein in three of 12,674 adequate tissue samples studied. First, because the stage of vCJD infection during which the appendix first accumulates detectable amounts of abnormal prion protein is not known and because the accumulations might not be uniformly distributed throughout the tissue, the prevalence of infection might have been underestimated. Second, because the study design (lacking examination of a large number of similarly obtained appendices from a non-BSE-epidemic country) did not permit an estimate of specificity of the method or an independent confirmation of results, it is possible that the results might have been false positives leading to an overestimation of prevalence. In their paper the authors stated: "Although immunohistochemical accumulation of PrP in lymphoreticular tissues has not been demonstrated in any disease other than vCJD, the significance of the positive samples in this study is not certain. In one case, the immunohistochemical pattern of immunoreactivity resembled that seen in appendix tissue from pre-clinical and autopsied cases of vCJD, but in the other two cases, a more finely granular pattern of staining was present in relation to follicular dendritic cells, raising the possibility that these may be false positives. However, we have been unable to demonstrate PrP immunoreactivity in a range of other disorders including other human prion diseases, neoplastic disease, or a range of inflammatory conditions."

**Assumption used in the model:** All vCJD cases that occur after 2002 are incubating in year 2002.

Prevalence of vCJD among the UK population and the vCJD risk from using plasma-derived factor products are expected to be different from year to year since 1980. In this risk assessment, the potential vCJD risk for pdFVIII products was estimated for the baseline year of 2002, but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. Prevalence of vCJD in 2002 for a specific age population in the UK was extrapolated from two estimates of prevalence discussed above based on age information of reported vCJD cases. The prevalence derived from above two different approaches varied by approximately 130 fold. The discrepancy reflects the limitation on the current knowledge of the disease. In order to evaluate the impact of uncertainty in estimation of vCJD prevalence, the FDA risk assessment provides estimated risk outcomes stratified by two estimates of prevalence.

**Assumption used in the model:** Our model assumed that distribution of asymptomatic cases across age groups would be the same as the distribution of observed symptomatic cases.

Additional technical information and details of analyses and modeling approaches are provided in Appendix A under section **A - IV.A.**

## IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2)

The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model.

This module estimates the annual number of plasma pools that are used to manufacture pdFVIII from plasma collected in the United States, the number of pools that potentially contain a donation from an infected plasma donor, and the potential quantity of vCJD agent that may be present in a positive pool. The potential vCJD risk for US plasma donors is likely associated with dietary exposure to BSE agent during periods of travel or residence in the at-risk geographic areas where BSE occurred. The percentages of blood donors with a history of travel or residency in BSE countries, who are military members who resided in bases in UK and elsewhere in Europe during 1980-1996, and who are recipients of "Euroblood" were obtained from 1980-1996 Blood Donor Travel Survey conducted by American Red Cross (TSEAC 2000). The percentage was calculated by destination (e.g., the UK, France or other European countries) and duration of travel.

Two different types of plasma are used in manufacture of pdFVIII. Source Plasma is collected through plasmapheresis, a process that separates red blood cells from plasma and returns red blood cells to the donor. Recovered plasma is prepared from whole blood units collected from blood donors. Source Plasma accounts for approximately 80% of the total plasma collected annually in the United States, and

recovered plasma accounts for the remaining 20%. Source Plasma donors are usually younger than blood (recovered plasma) donors, and are thought to travel less so presumably their vCJD risk may be somewhat lower than that of blood donors. However, because of their younger age demographic, Source Plasma donors are likely to be more susceptible to vCJD infection. Additionally, Source Plasma pools are usually smaller and contain larger volume donations (an average of 700 milliliters) from fewer donors than recovered plasma pools (average volume of a donation is ~200 milliliters). Plasma from fewer donors reduces the chance that a plasma pool may contain a donation from an infected donor. However, because Source Plasma donors are allowed to donate more frequently, and give more plasma per donation, there is a greater chance that if a vCJD infected donor were in the Source Plasma donor pool that they may contribute multiple donations to a single plasma pool or donate to multiple pools. However, blood deferral policies instituted beginning in 1999 are believed to have reduced the risk of vCJD donations by more than 90%. The effectiveness of the deferral policy in removing potential vCJD risk from the donor and donation pool is included in the FDA model. Because of the unique characteristics and potential differences in risk for Source and recovered plasma donations and plasma pools, the FDA risk assessment modeled Source and recovered plasma pools separately, and considered factors that may result in different risk for pdFVIII product made from each of the two types of plasma.

## IV. B. 1. a. Annual US plasma donors and characterization by age

Age is an important factor in estimating potential vCJD risk for US plasma donors. The FDA model is organized by age groups 18 and 19 yr olds, 10-14, 15-29, etc. (by five yr age groups to age 69) and calculates all risk information and makes all adjustments based on age groups. Each of these age groups forms a "bin" and in each of these bins donors are categorized by country of travel, vCJD prevalence (or relative risk) for country of travel, duration of travel, year of travel, type of donation (Source or recovered), donation rate, etc. The output at the end of this portion of the model is an estimation of the number of US donors in each age group that are potentially infected with vCJD. The model further incorporates the effect of FDA donor deferral policies, implemented beginning in 1999, that are believed likely to reduce the possible risk from blood donors potentially infected with vCJD by ~ 90%.

This specific portion of the model estimates potential age specific vCJD risk for both Source and recovered plasma donors. As mentioned above, donor age is an important factor associated with frequency of travel and susceptibility to the disease and influences the vCJD risk for a particular type of donor. For instance, Source Plasma donors as a group are generally younger than recovered plasma donors (see percentages of donors for Source and recovered plasma donors by age group in **Table 4.3**). Also, the younger Source Plasma donor population likely travels less, and thus, likely has a lower potential vCJD risk. However, this lower risk may be offset by the possibility that younger persons may be more susceptible to infection by the vCJD agent. The purpose of this portion of the model is to characterize plasma donors and their donations according to donor age to more accurately estimate the number of potential vCJD infected donors and donations containing vCJD agent. In turn this information will be used to estimate the probability that a plasma pool used in the manufacture of pdFVIII may contain a donation with vCJD agent.

Additional technical information and details of analyses and modeling approaches are provided in Appendix A under section **A - IV.B.**

**Table 4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups**

| Age group | <10 | 10-14 | 15-19 | 18-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | >70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reported vCJD cases in UK (through 2003)[a] (%) | 0 | 5 (3.4%) | 27 (18.4%) | | 32 (21.8%) | 30 (20.4%) | 22 (14.9%) | 13 (8.8%) | 5 (3.4%) | 3 (2%) | 5 (3.4%) | 0 (0%) | 5 (3.4%) | | |
| Age distribution of US Source Plasma donors (%)[b] | 0 | 0 | 0 | 12% | 29.3% | 14.1% | 14.1% | 9.6% | 9.6% | 5.8% | 5.8% | 0% | 0% | 0% | 0% |
| Age distribution of US Blood (Recovered plasma) donors[c] | 0 | 0 | 0 | 5% | 13% | 8% | 10% | 12% | 13% | 12% | 11% | 7% | 4% | 5% | 0% |

[a]Hilton *et al.* 2004

[b]Plasma Protein Therapeutics Association (Jan 07, 2005). Where data were organized in broader age group we allocated donor equally among smaller 5 year age groups

[c] Data provided to FDA by Westat in 2002

## IV. C. Estimation of the probability that a plasma pool may contain a donation from an infected donor that contains vCJD agent

The purpose of this section of the model is to estimate the prevalence of vCJD in US donors who may have been exposed to the BSE agent and potentially infected with vCJD during travel, residence, military service in the UK, France or other countries in Europe since 1980. The vCJD prevalence in US donors is then used to estimate the probability that a plasma pool may contain a donation from a vCJDinfected donor with infectious agent in their blood at the time of donation.

The starting material for manufacturing pdFVIII is a plasma pool containing donations from thousands of donors. The probability that a plasma pool contains a donation with vCJD agent is a function of the prevalence of vCJD in the US donor population and the total number of donors and donations present in a pool used to manufacture pdFVIII. For US donors with a history of travel, a key factor in estimating the vCJD risk is the region or country of travel, i.e., the UK, France or other countries in Europe, since 1980. From there we make several adjustments to the vCJD prevalence for US donors. The model incorporates the age of donors into the estimate of vCJD prevalence – donation rate for various age groups is important since the majority of donors are less than 40 years of age. Furthermore, vCJD prevalence for each age group is determined. Since vCJD primarily affects younger persons (median age 28 yrs) and donors are younger – they are at particular risk for vCJD and may unknowingly transmit the agent via donations. The model further adjusts vCJD prevalence based on the year of travel – for instance, a traveler in 1992 that visited the UK at the height of the BSE epidemic faces a higher BSE exposure risk and risk of vCJD infection than someone who traveled to the UK in 1997 after more stringent food controls were implemented in the UK. Also, the model incorporates information on the

duration of the travel based on survey information of travel history for whole blood users. Also considered in the model is the type of donation – whether Source Plasma or for recovered plasma, since the number of donations by an individual per year varies dramatically for these two types of plasma. Finally, the model assumes that *a plasma donation from a vCJD infected individual contains the infectious vCJD agent*, and thus poses a potential risk, if the individual is in the last half of the incubation period for the disease (and likely prionemic). However, current deferral policies prevent potentially infected donors with a history of extended travel or residence to BSE countries since 1980 from donating blood. *This geographic deferral policy effectively lowers the prevalence of vCJD in the US donor population by removing donors with a history of extended travel or residence in the UK and other countries in Europe since 1980.* The FDA risk assessment incorporates the effectiveness of current geographic deferral policy in reducing the risk of vCJD transmission through plasma derivatives. The final outputs or results from the model offer estimates of the potential number of Recovered and Source Plasma donors who may be infected with vCJD, and the model further derives an estimate of the number of infected donors who may actually have agent in their blood at the time of donation.

FDA evaluated a number of possible sources of exposure to the BSE agent that could potentially result in infection with vCJD in US plasma donors. The model and risk assessment assumes that the greatest potential vCJD risk for US plasma donors was likely associated with exposure to the BSE agent during extended travel or residence in the United Kingdom (for 3 months or more from 1980 – 1996), or France and other countries in Europe (5 or more years since 1980).

The following sections in the document describe in detail our mathematical approach for modeling and estimating the risk for US plasma donors who lived or resided in:

- United Kingdom from 1980 – 1996 for $\geq$ 3 months (Described in Appendix A - section A-IV.C.1.a.)
- France - since 1980 for $\geq$ 5 years (Described in section IV.C.1.b. and in Appendix A – sections in A-IV.C.1.b.)
- Other countries in Europe – since 1980 for $\geq$ 5 years (does not include plasma donors) (Described in section IV.C.1.c. and in Appendix A – sections in A-IV.C.1.c.)
- US Military personnel or their dependents – deployed in UK or other countries in Europe since 1980 (Described in section IV.C.1.d. and in Appendix A – sections in A-IV.C.1.d.)
- Euroblood recipients in US – that received blood collected from donors in Europe (Described in section IV.C.1.e. and in Appendix A – sections in A-IV.C.1.e.)

Dietary exposure in the US through consumption of domestic beef potentially contaminated with BSE agent was considered negligible based on our calculations, and was not included in the final model of this risk assessment.

**Assumption used in the model:** The BSE exposure risk for an individual on extended travel or during residence to the UK, France, or other countries in Europe since 1980 is proportional to the duration of the stay or time spent. For instance a person who lived in the UK for one year has one-fifth the risk of a donor who spent five years.

## IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood

The model considered all major potential sources of vCJD infection for US plasma donors. The most likely cause of vCJD is dietary exposure of donors to the BSE agent through the consumption of BSE-contaminated beef during travel to a country where BSE was present in the cattle population. Accordingly, the greatest risk of BSE exposure in the UK likely occurred during the period from 1980 to 1996. The BSE exposure risk for France was likely lower than that of the UK and likely present since 1980. Also, other countries in Europe likely posed an even lower risk than France of human exposure to the BSE agent since 1980. Generally, because of the higher prevalence of BSE in the UK in the late 1980s and early-to-mid-1990s and the higher occurrence of vCJD in the UK human population (currently 162 cases as of August 2006), US donors who traveled to the UK from 1980 through 1996 are likely at higher risk for vCJD infection than donors who traveled to other European countries in the same time period. This model uses the concept of relative risk to estimate the vCJD risk (and prevalence) for a donor population – a value of 1 is used for the UK and this is equal to the vCJD prevalence. Relative risk is used to compare the risk of other regions to that of the UK and is estimated based on factors such as amount of contaminated feed, percentage of meat from the UK, number of cases of BSE, vCJD, etc. In subsequent sections on estimating risk for donors that traveled to France, France is assumed to have a relative risk of 0.05, since they received about 5% of their beef and feed supply from the UK and also have reported domestically-acquired vCJD cases.

The potential vCJD risk faced by US plasma donors exposed to vCJD during travel or residence in the UK (since 1980) is assumed in the model to be proportional to the time a donor spent in the UK (or France or other countries in Europe), and also a function of the age of the donor, and year of travel. Duration of travel is an indicator of possible exposure and we assumed that the probability of exposure was proportional to the time spent in the UK from 1980 - 1996. The longer the duration of travel, the higher the risk of human exposure to the BSE agent. The magnitude of possible exposure to the BSE agent is also influenced by the specific year of travel. The risk is the highest when travel took place during the peak of BSE epidemic in 1992. The FDA risk assessment grouped plasma donors based on age, destination, duration and year of travel, estimated the number of donors, probability of an individual being infected, and potential number of infected donors for each group. Then, numbers of infected donors from all groups were summed to arrive at an estimate of the total number of potential vCJD infected donors in the US that may contribute to FVIII plasma pools. The vCJD agent may only be present in the blood of an infected person during the last half of the disease. Accordingly, the FDA risk assessment considered only those vCJD infected donors who were in the last half incubation period of the disease as being capable of possibly transmitting the disease to others through their plasma donations.

The FDA risk assessment evaluates the effectiveness of current geographic deferral policy in reducing the risk of vCJD transmission through plasma derivatives. Current policy defers individuals with history of long term travel in BSE epidemic areas since 1980, providing a barrier for donors potentially infected, thus effectively lowering the vCJD prevalence in the US donor population by an estimated 90% or more. However, there may be residual risk associated with donors who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process. Although current policy defers US donors that received transfusions while in the UK or France since 1980, the FDA risk assessment did not estimate the potential vCJD risk for these donors.

**Assumption used in the model**: vCJD risk for the US plasma donor population is a sum of the risk from all exposure sources.

**Assumption used in the model**: The FDA risk assessment assumed vCJD agent is present in the plasma of infected person only in the last half of the incubation period of the disease, based on animal studies on vCJD infectivity of blood.

**Assumption used in the model**: The mean incubation period for vCJD is 14 years, and the median incubation period is 13 years.

The United Kingdom has the highest number of reported BSE and vCJD cases in the world. US plasma donors that traveled to the UK during 1980-1996 are considered at risk of exposure to the BSE agent and possibly infected with vCJD. Donors that traveled to France and other countries in Europe are assumed to be at significantly lower risk since the BSE epidemic in those regions was many times smaller than in the UK. This part of the model further characterizes the plasma donors of each age group who have history of travel or residence to the UK (for $\geq$ 3 months from 1980 – 1996), and France or other countries in Europe (for $\geq$ 5 yrs since 1980), by duration and calendar year of travel, estimated the number of potential donors who may be infected, and number of donor who may be in the last half incubation period (prionemic) at year 2002.

## IV. C. 1. a. US plasma donors with history of travel to the UK: Number of donors potentially infected and vCJD agent is present in the blood

This portion of the model calculates the potential vCJD risk for US plasma donors who traveled to the UK since 1980 and estimates the potential number of donors with vCJD agent present in their blood and donated to plasma pools used to manufacture pdFVIII. For blood and plasma donors with a history of travel to the UK FDA guidance (2002) currently recommends that blood and plasma collection centers "....defer blood and plasma donors who have traveled or resided in the U.K. for a cumulative period of 3 or more months between 1980 and the end of 1996." The deferral policy likely eliminates much approximately 90% of the potential vCJD risk associated with donor travel to BSE countries when the disease was present. The model incorporates the effectiveness of the deferral policy in reducing risk. However, residual vCJD risk remains from two sources. One source includes potential risk associated with deferrable donors that continue to donate because of limitations in the donor screening process. The second potential source is associated with donors with a history of travel that is less than the deferrable period (blood or plasma donors with a history of less than 3 months of travel to the UK).

In addition to the effectiveness or deferral policies, the residual risk of vCJD infection in US donors is calculated based on the proportion of time spent in the UK by US donors compared to a UK resident whose risk is equivalent to the UK vCJD prevalence. Additionally, the model considers factors such as calendar year of travel, age of donor, type of donation (Source or recovered plasma), possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US plasma donors who were potentially infected with vCJD during travel to the UK (see Table 1). The model also predicts the

number of donors that potentially have agent present in their plasma that may possibly donate to plasma pools used to manuafacture pdFVIII in the US.

Additional technical information and details on the model calculations of potential vCJD risk and modeling approaches are provided in Appendix A and described in sections in A - IV.C.

## IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

The FDA risk assessment model assumes that the likely source for vCJD risk in US plasma donors is associated with those donors who have a history of travel to the UK from 1980 – 1996, or travel to France or other countries in Europe since 1980. Presumably the greatest vCJD risk resides with travel to the UK since the BSE epidemic was several orders of magnitude larger and the number of vCJD cases greater than that of any other country or region. However, donors who traveled to France are potentially at risk but that risk is likely significantly lower than that of the UK. France likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 5% of its beef was supplied by the UK at the time of its BSE epidemic. To date, France has reported 20 cases of vCJD supporting the notion that there may be vCJD infection risk for US donors that may have traveled to or resided in France since 1980.

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) to estimate potential vCJD risk for donors with a history of travel to countries where BSE has occurred. FDA guidance (2002) indicates that for donors with a history of travel to France "we now recommend deferral of blood and plasma donors with a history of 5 or more years of cumulative residence or travel in France since 1980." This portion of the model calculates the potential residual US vCJD risk based on the estimated vCJD prevalence among US plasma donors who traveled to France since 1980 and donated to plasma pools used to manufacture pdFVIII. The residual vCJD risk includes risk associated with donors who do not meet criteria for donor deferral (e.g., donors with a history of less than 5 years of travel to France), or who meet those criteria, but fail to be deferred due to limitations of the donor screening process. The model considers factors such as duration of travel, calendar year of travel, age of donor, type of donation (Source or recovered plasma), possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US plasma donors potentially infected with vCJD and results are shown in Table 4.4. The model goes on to predict the number of donors with a history of travel to France infected with vCJD that may potentially have agent present in their blood at the time of donation.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of travel to France can be found in Appendix A – in sections under A-IV. C.1.b.

## IV.C. 1. c. Number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) potentially infected and vCJD agent is present in the blood

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) in estimating potential vCJD risk for donors with a history of travel to countries where BSE has occurred. The model quantifies potential residual vCJD risk for US plasma donors. The model assumes that the residual vCJD risk likely originates from two sources, one source of potential risk is associated with donors with deferrable risk that continue to donate due to limitations in the donor screening process. The second source of vCJD risk is associated with donors with a history of travel that was less than the deferrable period (blood donors with a history of less than 5 years of travel to Europe). FDA guidance (2002) indicates that for donors with a history of travel to countries in Europe (other than the UK) "the current recommendation is to exclude from transfusion use, blood and blood components from donors with a history of 5 or more years of residence or travel in Europe outside of the UK". Furthermore, for donors with a history of travel to countries in Europe (other than the UK and France) the FDA guidance (FDA 2002) states "...we do not recommend that you defer Source plasma donors who have lived or traveled in Europe for 5 or more years".

The FDA risk assessment model reflects FDA guidance for vCJD deferral of Source Plasma and recovered plasma donors. Because the guidance recommendations for each type of plasma were different the model estimated the potential vCJD risk as follows:

- Recovered plasma donors – the FDA risk assessment **calculated** the potential vCJD risk because deferral was **recommended** for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK)
- Source Plasma donors - the FDA risk assessment did **not calculate** the potential vCJD risk because deferral was **not recommended** for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK and France).

The term "countries in Europe" as used in this portion of the risk assessment is defined as all countries in Europe (other than the UK and France). The UK and France BSE risk is assumed to be higher than that of other countries in Europe, therefore the potential donor vCJD risk for donors with a history of residence or travel to the UK and France described in sections IV.C. 1. a. and IV.C. 1. b.(and sections in Appendix A – under sections A-IV.C. 1. a. and A-IV.C. 1. b.), respectively, of this document. Also, US donors with a history of residence on US military bases in Europe may have a higher potential vCJD risk so their risk was calculated separately and is described in section IV.C.1.d.

This portion of the model calculates the vCJD prevalence, or number of US blood donors who traveled to other countries in Europe (other than the UK and France) since 1980 and were potentially infected with vCJD and donated to recovered plasma pools used to manufacture pdFVIII. Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. The model considers factors such as duration of travel, calendar year of travel, age of donor, type of donation, possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US blood (recovered plasma) donors who are potentially infected with vCJD and have agent present in their plasma.

The model assumes that one of the most likely sources for vCJD risk in US plasma donors is associated with those donors who have a history of travel to the UK from 1980 – 1996, and France and other countries in Europe since 1980. Text in subsequent sections of this document will specifically discuss the potential vCJD infection risk for US plasma donors with a history of travel to countries in Europe (other than the UK and France) since 1980. Presumably the greatest vCJD risk resides with travel to the UK since 1980 since the BSE epidemic was several orders of magnitude larger and the number of vCJD cases greater than that of any other country or region. France is likely at significantly lower risk than the UK but the risk is still likely higher than the vCJD risk of other countries in Europe. There have been 20 cases of vCJD reported in France as of August 2006.

Donors who traveled to other countries in Europe (other than the UK or France) are potentially at risk but that risk is likely significantly lower than that of the UK, and France. Many European countries (other than the UK and France) likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 1.5% of their beef may have been imported from the UK at the time of its BSE epidemic. The potential for BSE exposure to donors who traveled to or resided in other countries in Europe is possible. Hence, there may be a vCJD infection risk for blood donors who may have traveled to or resided in a European country (other than the UK and France) for periods greater than 5 years since 1980. The current US vCJD geographic deferral policy defers blood donors with a history of travel or residence in a country in Europe (other than the UK and France) for 5 years or more since 1980. Source Plasma donors who resided in a country in Europe (other than the UK and France) are not deferred from donation. Because Source Plasma donors are not deferred from donation, their risk is not estimated by the model. Therefore, this portion of the model only estimates potential vCJD risk for US recovered plasma donors who traveled to countries in Europe (other than the UK) since 1980.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of travel to countries in Europe (other than the UK and France) can be found in Appendix A – in sections under A-IV. C.1.c.

## IV. C. 1. d. Number of US plasma donors deployed by the military in the UK or other countries in Europe and potentially infected with vCJD

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) in estimating potential vCJD risk for donors with a history of travel to countries where BSE has occurred. The FDA guidance (2002) indicates that for donors with a history of service on US military bases in Europe "we recommend that you should indefinitely defer current and former US military personnel, civilian military personnel, and their dependents who were stationed at European bases for 6 months or more during the time periods outlined (in the document)".

The model quantifies potential residual vCJD risk for deferrable donor populations. The residual vCJD risk likely includes two possible sources of risk. One source of risk is associated with donors with deferrable risk that continue to donate because of limitations in the donor screening process. A second possible source of risk is associated with donors who have a history of travel that is less than the deferrable period (blood donors with a history of less than 5 years of travel to Europe). For the purposes of this risk assessment we assumed that exposure to the BSE agent and potential vCJD infection of military personnel or dependents may have occurred during their deployment to US military bases in the UK, France and other European countries during the period from 1980 through 1996, through consumption of BSE contaminated beef procured for use on US military bases from the UK.

Exposure via UK beef likely varied but the model assumes that up to 35% of beef consumed on military bases in Europe came from the UK. The model assumes that approximately 2% of US blood and plasma donors may have been military, military family or their dependents posted to US military bases in the UK or elsewhere in Europe from 1980 through 1996 (TSEAC, 2002). It was further assumed that the average deployment period was 2 years. Because data on military service of plasma donors was not available, the risk assessment used data available on military deployment by whole blood donors.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of military service in countries in Europe can be found in Appendix A – in sections under A-IV. C.1.d.

## IV. C. 1. e. Annual number of US plasma donors who have been Euroblood recipients

Euroblood is whole blood that was collected at several different collection centers in Europe and shipped to and used by transfusion centers in the United States. The practice was stopped in 2002 with the implementation of geographic vCJD deferrals. The blood was used largely on in the New York City metropolitan area and possibly in other areas on the east coast of the US

The model assumed that a total of 1.2% of US blood donors may have received Euroblood (TSEAC, 2002). To our knowledge there are no specific data available for plasma donors, therefore, data for blood donors was used in this risk assessment.

**Assumption used in the model:** All infected Euroblood recipients have vCJD agent present in their blood and plasma (prionemic)

## IV. C. 1. f. Total number all plasma donors who may potentially be infected with vCJD through all sources of exposure and vCJD agent is present in the blood

This portion of the model sums the total number of all potential US donors that may have been infected with vCJD from different sources. The model estimates the total number of all plasma donors who may be infected with vCJD during extended residence, travel or military service in the UK, France, or other countries of Europe. Potential vCJD risk is also estimated for donors that may have received Europblood. Furthermore, the model estimates the number of total US donors potentially infected with vCJD and have agent present in their plasma.

**Variable:** $\mathbf{DR_{vCJD\text{-}Pn}}$ - Total annual number of <u>all</u> US plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-Pn} = DR_{vCJD-S-Pn} + DR_{vCJD-R-Pn} \qquad \text{(IV.C.1.f-3)}$$

Additional technical information and details of analyses and modeling approaches for estimating potential vCJD in the model are provided in Appendix A under section **A - IV.C.1.**

## IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in the blood and who may not be deferred by questionnaire screening

No validated test is currently available to detect the presence of vCJD agent in blood or plasma. The donor questionnaire, administered to all blood donors, can be used to potentially screen donors for potential vCJD risk based on travel history, specifically involving extended travel to the UK, France or other countries in Europe where BSE was known to occur. In 1999 the FDA implemented a donor deferral policy aimed at reducing the potential risk of donations from those potentially exposed to the BSE agent during extended travel to the UK, France and other countries of Europe. Current policies (FDA 2002) defer blood and plasma donors:

- diagnosed with vCJD or other forms of CJD
- at increased risk for CJD, e.g. the donors have received a dura mater transplant, or human pituitary-derived growth hormone; the donors have blood relatives diagnosed with CJD
- with a history of a 3-month or longer travel/residency period in the UK between 1980-96
- with a history of a 5-year or longer travel/residency period in France since 1980
- current or former US military personnel, civilian military personnel, and their dependents resided in Northern Europe for 6 months or more between 1980-90, or resided in military bases elsewhere in Europe for 6 months or more from 1980 to 1996
- received a transfusion of blood or blood components in the UK since 1980
- injected bovine insulin since 1980 unless it is confirmed that injected bovine insulin was not made after 1980 from UK cattle, and
- whole blood donors with a 5-year or longer travel/residency period in Europe (other than the UK) since 1980

Deferral of donors with a history of travel to BSE countries is an effective tool for eliminating a significant portion of potential vCJD risk in US donors. The model incorporates information on the effectiveness of US deferral policies in reducing potential vCJD risk and potential vCJD prevalence in the US donor population.

**Assumption about variable**: Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Assumption about variable**: Model includes potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) that have long term travel history to the UK (≥3 mo), France (≥ 5 yrs), and Europe (≥5 yrs); and history of military deployment, military dependent or related travel or residence in Europe.

There is a possibility that some individuals that traveled to the UK, France, and other countries in Europe since 1980 stayed for periods of time that were shorter than the deferral period, were exposed to BSE agent, and were infected with vCJD. These individuals represent a source of residual risk – or the remaining risk after interventions (in this case donor deferral policies) are applied. The section below addresses the calculation of residual risk for non-deferred at risk donors that traveled for periods of time that were shorter than recommended guidelines.

The total number of all US plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that may not be deferred by questionnaire screening was determined by summing the estimates generated for both Source and recovered plasma donors that may not be deferred by current screening procedures.

Specific technical information and details of analyses and modeling approaches in estimating the effectiveness of donor deferral policies in reducing potential vCJD risk in US donors are provided in Appendix A under section **A - IV.C.2.**

**Model Results:**
The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model.

The FDA FVIII risk assessment model uses the concept of relative risk to semi-quantitatively estimate the vCJD risk for US plasma donors with a history of travel to the UK, France and other countries of Europe since 1980. Relative risk is the vCJD risk in a population relative to the UK vCJD relative risk of 1 (or 100%), which is equal to the prevalence of vCJD in the UK. Elements used in the model to calculate vCJD risk for travelers include travel destination (UK, France or other countries of Europe), duration of travel, specific year of travel, and age of donor. The estimated vCJD risk for all potential routes was summed to generate the total mean predicted number of potential vCJD-infected plasma donors in the US. Because of current policies, a blood or plasma donor potentially infected with vCJD has a high probability (85% - 99% chance) of being deferred from donation. Since 2002 FDA has recommended that individuals who resided in the UK for a period of 3 months or more from 1980 to 1996, or resided in France or other countries of Europe for a period of 5 years or more since 1980 be deferred from donating blood or plasma. The model assumes the deferral policy in the US is approximately 85% to 99% effective in reducing the vCJD risk for blood and plasma-derived products. **Table 4.4** (below) shows results from the model predicting the mean number per year of potential vCJD-infected donors and the number of potential vCJD donors who are likely not deferred from donation and donate to plasma pools used to manufacture pdFVIII.

Although it is likely that most would be deferred by the current policy, some plasma donors potentially infected with vCJD may not be deferred and may donate to plasma pools used in the manufacture of pdFVIII. Totaling the estimated number of US donors potentially infected with vCJD yields a mean of approximately 0.01 donors per year based on calculations using a vCJD case-based epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani 2005), or a mean of approximately 1.160 donors per year using calculations based on a tissue sample surveillance study yielding a prevalence estimate of 1 in 4,225 (Hilton *et al* 2004) (**Table 4.4**).

**Table 4.4 Model Results: Annual Number of US plasma donors predicted by model to be potentially infected with vCJD and donate to plasma pools used to manufacture pdFVIII.** Results from model provided for two different UK vCJD prevalence estimates. In the table the mean value is shown above with the 5th and 95th percentiles in parentheses below. The total number of vCJD donors for each prevalence estimate has been rounded to nearest decimal place.

| | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* | |
|---|---|---|---|---|
| | **Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:** | | **Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:** | |
| | **United Kingdom** | ***France, Europe, or*** **Military Service** | **United Kingdom** | ***France, Europe, or*** **Military Service** |
| ***Total number vCJD donors for all US pdFVIII pools Prior to screening*** | *0.0493* (0– 0)[b] | *0.0108* (0– 0)[b] | *5.32* (0– 13) | *0.77* (0 – 3) |
| **Number vCJD donors NOT DEFERRED** (ineffective screening) | *0.0035* (0– 0)[b] | *0.0007* (0– 0)[b] | *0.39* (0-2) | *0.056* (0– 0)[b] |
| ***Number vCJD donors NOT DEFERRED*** *(short-term travel <3 mos, UK; <5 yrs, FR and EU)* | *0.0049* (0– 0)[b] | 0.0017 (0– 0)[b] | *0.483* ( 0 – 2) | 0.0343 (0– 0)[b] |
| ***Total number vCJD infected donors NOT DEFERRED Donate to pdFVIII Plasma Pools*** | ***0.013*** *(0 – 0)[b]* | | ***1.160*** *(0 – 4)* | |

[a] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## IV. D. Annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US

***Model Results:***
The percentages of source or recovered plasma pools, potentially containing vCJD agent, used to manufacture pdFVIII in the US were estimated by the model. The majority of pdFVIII is manufactured from Source Plasma and the minority from recovered plasma. Manufacturers provided information to FDA on the approximate range and average number of donations per plasma pool which was combined with information on market share to develop two aggregate statistical distributions, one each representing donations for source and for recovered plasma pools. The distributions were used to predict the number of donations per source or recovered plasma pool in the model. The model used information on the number of donations per pool by type (either source or recovered), combined with estimated yield of pdFVIII per pool, and estimated the total number of plasma pools used to manufacture pdFVIII products distributed in the US in 2002.

As a general comment, the number of donations per plasma pool influences the potential exposure risk for infrequent recipients of plasma derivatives. The use of fewer donations and smaller plasma pools during manufacturing would result in a lower percentage of plasma pools potentially containing vCJD agent and potentially expose a lower percentage of infrequent recipients to vCJD (if present). Frequent recipients of plasma-derived products would likely face a similar level of risk of potential vCJD exposure whether large or small numbers of donations per plasma pool are used in manufacturing.

*Lower UK vCJD prevalence estimate of ~1.8 in 1,000,000 (based on Clarke and Ghani, 2005).* The lower prevalence estimate used in the FDA model suggested that an average of 0.027% of all US plasma pools used to manufacture pdFVIII in the year 2002 potentially contained the vCJD agent (bottom, **Table 4.5**). The lower disease prevalence is associated with model results predicting a much lower percentage of plasma pools potentially containing vCJD agent. In fact, on average >99.9% of the time plasma pools would be predicted not to contain a donation from a vCJD infected donor. Only an average 0.10% recovered plasma pools would be predicted by the model to contain a vCJD donation from a US donor in any given year. Of interest at the lower prevalence, the model predicts that the occurrence of a recovered plasma pool with a vCJD donation would be infrequent (as indicated by 5$^{th}$ and 95$^{th}$ percentile values of 0); occurring (as estimated by the model), at a rate of 1 in 100 years. Also at the lower prevalence, a vCJD donation in a Source Plasma pool would be predicted to be even more infrequent and predicted by the model to occur at a rate of 1 in 200 years.

*Higher UK vCJD prevalence estimate of 1 in 4,225 (Hilton et al 2004).* The higher prevalence estimate used in the FDA model suggested that an average of 2.41% of all US plasma pools used to manufacture pdFVIII in 2002 were predicted by the model to contain vCJD agent (**Table 4.5**). It should be noted that fewer recovered plasma pools than Source Plasma pools are used in the US annually to produce pdFVIII. Also, recovered plasma pools contain the largest number of plasma donations. Since recovered plasma pools

contain many more donations than Source Plasma pools the likelihood that a recovered plasma pool may contain a donation from an individual potentially infected with vCJD is considerably higher than for a Source Plasma pool. Using the higher UK vCJD prevalence estimate, the model predicts that on average, 9.12% of recovered pools and 0.96% of Source Plasma pools potentially contain vCJD agent.

**Table 4.5 Annual Percentage of US Plasma Pools Potentially containing a vCJD Donation.** Results from model include only those US plasma pools used annually to manufacture pdFVIII.

- Results provided for two different UK vCJD prevalence estimates.

| | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton, et al (2004) | |
|---|---|---|---|---|
| | **Source** Mean ($5^{th}$ - $95^{th}$ perc)[a] | **Recovered** Mean ($5^{th}$ - $95^{th}$ perc)[a] | **Source** Mean ($5^{th}$ - $95^{th}$) perc)[a] | **Recovered** Mean ($5^{th}$ - $95^{th}$ perc)[a] |
| **Percent pools potentially containing vCJD agent** | 0.01% (0 – 0%)[b] | 0.10% (0 – 0%)[b] | 0.96% (0 – 5.88%) | 9.12% (0 – 40.17%) |
| **Average percent pools potentially containing vCJD agent** | **0.027 %** (0 – 0 %)[b] | | **2.41 %** (0 – 10 %) | |

[a] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[b] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

Additional technical details on the calculation of the annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US are provided in Appendix A in sections under A-IV. D.

## IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD

Based on limited available data (see below), FDA believes that the quantity of infectivity present in blood from a vCJD infected individual in i.v. $ID_{50}$ is likely represented by a distribution with the following characteristics: Minimum value = 0.1, 5$^{th}$ percentile = 2, Most likely value = 10, 95$^{th}$ percentile = 30, and Maximum value= 1,000 i.v. $ID_{50}$. Given the possible parameters, statistical distributions were fitted to the selected parameters using Best Fit part of the @Risk Professional software package (Palisade Corporation, New York). Using the software we determined that a log normal statistical distribution of (2, 12, 30) i.c. $ID_{50}$/ml (5$^{th}$ percentile, most likely, and 95$^{th}$ percentile) with minimum and maximum of 0.1 and 1,000, respectively, provided the best fit.

Conclusions from several research groups arrive at somewhat similar estimates for the quantity of infectivity that might be present in the whole blood of mice and hamsters. Using a mouse model and human CJD Brown *et al* (1999) found a range from 0.5 to 15 mouse i.c. IU per ml which we assumed to be roughly equivalent to 1 to 30 i.c. $ID_{50}$ (assuming a linear dose-response for infectivity). An infectious unit is the quantity of infectivity associated with a 100% probability of infection in recipients and roughly equates to two $ID_{50}$ units (1 IU = 2 $ID_{50}$). Brown *et al* (1998, 1999) conducted experiments to determine the infectivity of buffy coat material and plasma but not red blood cells. Assuming that red blood cells retain approximately 25% of the infectivity of whole blood, then the infectivity present in whole blood could be estimated to be in the range of approximately 10 i.c. $ID_{50}$ and 20 i.c. $ID_{50}$ per ml. Cervenakova *et al* (2003) found levels as high as 20 – 30 infectious doses per ml (40-60 i.c. $ID_{50}$ per ml) associated with buffy coat and plasma during incubating and symptomatic stages of the disease. Red blood cells were not found to be infectious. Transfusion of blood products using the hamster scrapie model by Rohwer suggests that addition of infectivity levels derived for individual blood components would generate a titer for whole blood of approximately 2 to 20 i.c. $ID_{50}$/ml. Summarizing the above literature it seems that the range of reported values for infectivity ranged from 0.5 to as high as 30 i.c. $ID_{50}$ with the possibility that at times the infectivity present in blood may exceed this range.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5$^{th}$ percentile of 2 i.c. $ID_{50}$ per ml, a medium of 12 i.c. $ID_{50}$ per ml, a 95$^{th}$ percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing

less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected(Wadsworth, 2001).

## IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD

This section of risk assessment estimated quantity of vCJD agent in each vCJD plasma pool that may be used to make pdFVIII. Quantity of infectious agent present in plasma pools may vary depending on number of infected donations in the pool and the volume of each infected donation.

**Variable:** $I_{bl}$ – Represents the i.c. $ID_{50}$ present in the blood of individual infected donor ($ID_{50}$/ml) in the last half of the incubation period of vCJD.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5$^{th}$ percentile of 2 i.c. $ID_{50}$ per ml, a median of 12 i.c. $ID_{50}$ per ml, a 95$^{th}$ percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected (Wadsworth, 2001).
**Variable:** $I_{pl\text{-}perc}$ – Percent (%) i.v. $ID_{50}$s associated with plasma

Studies in animal models have shown that greater than 50% of transmissible spongiform encephalopathy agent present in whole blood is associated with plasma. Experiments by Gregori *et al.* (2004) using a hamster – sheep scrapie model showed that approximately 58% of infectivity in whole blood is associated with plasma.

**Assumption used in the model:** The model assumes that 58% of infectivity is associated with plasma.

Studies with mouse-adapted scrapie agent suggest that the i.v. route of administration is approximately 10 times less efficient in causing infection than the intracerebral route (Kimberlin *et al* 1996). Brown *et al* (1999) used a mouse-adapted human TSE agent to show that i.v. injection of plasma was about seven times less efficient and i.v. injection of buffy coat approximately 5 times less efficient than were i.c. inoculations of the same materials in transmitting infection. Based on discussion and advice from the FDA Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC, 2005) the range of efficiency of the i.v. route (versus the i.c. route) was assumed in the model to range between the values of 1 and 10.

**Assumption used in the model:** Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

## IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD

The quantity of vCJD agent potentially present in a donation from a US donor infected with vCJD will be diluted out in a plasma pool among plasma from thousands of other donations. This section calculates the quantity of agent potentially present in a plasma pool potentially containing a donation that contained vCJD agent.

**Assumption used in the model:** We assumed only one infected donor per plasma pool, because based on the calculation in section IV. C. 5. the prevalence of vCJD in the US is very low and the chance a pool involves multiple donations from vCJD infected donors is small.

**Variable:** $DN_{DR}$ - Number of donations from an infected plasma donor, which varies based on type of plasma donated.

**Assumption used in the model:** We assumed individual infected Source Plasma donor most likely give -- donations to a pool, with minimum of 1, maximum of 12 donations. Individual infected recovered plasma donors most likely give only one donation to a pool.

**Variable:** $I_{Pool}$ - Initial infectivity in an infected plasma pool is represented by the equation:

$$I_{Pool} = I_{DN} \times DN_{DR} \qquad \text{(IV.E.2-1)}$$

## Module 3: Reduction in the quantity of vCJD agent during manufacture of pdFVIII

The plasma separated from whole blood is a protein rich, straw-colored liquid that contains FVIII, a number of other clotting factors, immune globulins, serum albumin and a number of other proteins. Individual proteins, such as FVIII, can be purified by dividing or fractionating the plasma into its various protein components. Fractionation steps may include alcohol precipitation, size exclusion, affinity chromatography, etc. The quantity of vCJD agent present in plasma-derived products may be reduced through removal of the agent during the fractionation process. Although the quantity of agent may be significantly reduced, it may not be entirely eliminated.

Common viral inactivation procedures such as heating, solvent-detergent treatment, and UV irradiation have little effect on the quantity of TSE infectivity present in plasma and plasma derivatives. However, several research studies suggest that TSE infectivity in plasma may be partially removed during the fractionation process. The plasma fractionation procedures, which may remove vCJD infectivity, are summarized in **Table 4.6.** (Lee et al. 2000; Stenland *et al.* 2002; Foster 2004; Foster, *et al.* 2004).

For a specific pdFVIII product, usually only one or two processing steps have been studied for potential reduction of infectivity. Experimental designs of these studies are not standardized; therefore, study results are not directly comparable. In order to achieve a high concentration of vCJD infectivity in initial materials, many studies used vCJD infected brain homogenate as spiking material, which may not mimic the physical form of infectious agent in the blood. Considering the uncertainty in the degree of reduction that can be achieved during various pdFVIII manufacturing processes, this risk assessment models three levels of potential reduction in infectivity, 2-3 $\log_{10}$, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

**Table 4.6 Reduction factor (RF) of fractionation procedures**

| Fractionation Procedures | RF ($\log_{10}$ $ID_{50}$) | References |
|---|---|---|
| Cryoseparation | 0-1 | (Foster *et al* 2000; Farrugia 2002; Lee *et al* 2002) |
| General fractionation | | |
| 3% PEG | 1-3 | (Farrugia 2002; Lee *et al* 2002) |
| 11.5% PEG | 2-4 | (Farrugia 2002) |
| $Zn+Al(OH)_3$ | 1.7 | (Foster *et al* 2000) |
| Ion exchange | 2.7-3.5 | (Foster *et al* 2000; Cervenakova *et al* 2002) |
| Membrane filtration | 1 | (Foster *et al* 2000) |
| Immunopurification | 4.4-6.3 | (Foster, Welch *et al* 2000; Cervenakova *et al* 2002) |

## IV.E. 3. Model results: Estimates of the per vial vCJD infection risk for US manufactured pdFVIII (Module 3)

The mean potential risk of vCJD infection per 1,000 IU vial of US manufactured pdFVIII is shown in **Table 4.7.** The mean potential per vial vCJD risk per year is a function of two factors:

1) Percentage of pdFVIII vials containing vCJD agent and,
2) Quantity of agent (i.v. $ID_{50}$) present in each vial.

If vCJD agent were present in US plasma pools, the risk assessment model assumed that the quantity of agent was likely reduced by manufacturing processes used to produce

purified pdFVIII. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. **Table 4.7** shows potential risks associated with products attaining a 4-6 $\log_{10}$ level of reduction during manufacture. Results are shown only for 1,000 IU vials but the model assumed that the final purified pdFVIII product was packaged with equal likelihood into vial sizes of 250, 500, 1,000 and 1,500 international units (IU).

*Per vial vCJD risk: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani, 2005).* The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the lower prevalence estimate *(based on Clarke and Ghani 2005)* and results are shown in **Table 4.7**. Based on the lower prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 0.027%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. $ID_{50}$ per vial is $3.59 \times 10^{-5}$ for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 55,710 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 210 million. Alternatively, this could be taken to mean that a patient would need to infuse 210 million vials of product before accumulating one full infectious dose of vCJD.

At this lower prevalence estimate, there is a lower probability that plasma pools contain a donation from a donor potentially infected with vCJD, and a pdFVIII vial would be much less likely to contain vCJD agent. Although the probability (0.027%) is lower, the mean quantity of vCJD agent (iv $ID_{50}$) and risk of exposure to vCJD agent (1 in 55,710) per vial derived from a pool containing a single vCJD donation would likely be unaffected by prevalence. Readers may notice that the 5$^{th}$ and 95$^{th}$ percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, the model predicts that, on average, 0.027% of the time the exposure to vCJD may be greater than zero.

*Per vial vCJD risk: Results based on higher surveillance prevalence estimate of 1 in 4,225 (Hilton, et al 2004).* The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the higher prevalence estimate (Hilton, et al 2004) and results are shown in **Table 4.7**. Using the higher prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 2.41%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. $ID_{50}$ per vial is $3.59 \times 10^{-5}$ for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 55,710 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 2.3 million. Alternatively, this could be taken to mean that a patient would need to infuse 2.3 million vials of product before accumulating one full infectious dose of

vCJD. Again, although the probability (2.41%) is higher at the higher prevalence, the mean quantity of vCJD agent (iv $ID_{50}$) and risk of exposure to vCJD agent (1 in 55,710) per vial derived from a pool containing a single vCJD donation would likely be unaffected by prevalence.

**Table 4.7 Annual Predicted per Vial vCJD Infection Risk for US Manufactured pdFVIII from Model:**

- Results for 1,000 IU vial
- Assumed manufacturing process reduction of 4-6 $\log_{10}$ , and
- Two different UK vCJD prevalence estimates.

| | **4 - 6 $Log_{10}$ Reduction** | | | | | |
|---|---|---|---|---|---|---|
| | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | | | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton, et al (2004) | | |
| **Type of Plasma Pool** | **Percentage FVIII vials with vCJD agent** ($5^{th}$, $95^{th}$ perc)[c] | **Quantity iv $ID_{50}$[a] per vial*** ($5^{th}$- $95^{th}$ perc)[c] | **Mean potential per vial vCJDrisk[b]** ($5^{th}$- $95^{th}$ perc)[c] | **Percentage FVIII vials with vCJD agent** ($5^{th}$, $95^{th}$ perc)[c] | **Quantity iv $ID_{50}$[a] per vial*** ($5^{th}$- $95^{th}$ perc)[c] | **Mean** potential per vial vCJDrisk[b]** ($5^{th}$- $95^{th}$ perc)[c] |
| **Source** | **0.01%** (0 – 0%)[d] | **$4.36 \times 10^{-5}$** ($4.51 \times 10^{-8}$ – $1.43 \times 10^{-4}$) | **1 in 459 million** (0, 0)[d] | **0.96%** (0 – 5.88%) | **$4.36 \times 10^{-5}$** ($4.51 \times 10^{-8}$ – $1.43 \times 10^{-4}$) | **1 in 4.8 million** (0, 1 in 238,000) |
| **Recovered** | **0.10%** (0 – 0%)[d] | **$2.56 \times 10^{-6}$** ($3.13 \times 10^{-7}$ – $8.12 \times 10^{-6}$) | **1 in 800 million** (0, 0)[d] | **9.12%** (0 – 40.17%) | **$2.56 \times 10^{-6}$** ($3.13 \times 10^{-7}$ – $8.12 \times 10^{-6}$) | **1 in 8.6 million** (0,1 in 613,000) |
| **Average of all vials** | **0.027 %** (0 – 0%)[d] | **$3.59 \times 10^{-5}$** ($8.18 \times 10^{-7}$ – $1.29 \times 10^{-4}$) | **1 in 210 million** (0, 0)[d] | **2.41 %** (0 – 10 %) | **$3.59 \times 10^{-5}$** ($8.18 \times 10^{-7}$ – $1.29 \times 10^{-4}$) | **1 in 2.3 million** (0, 1 in 155,000) |

*Mean i.v. $ID_{50}$ in vials containing vCJD agent

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual per vial vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Percentage vials with vCJD agent x mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation

This section of the risk assessment models each infected plasma pool to estimate the potential reduction in infectivity for each pool during manufacturing processing, and to estimate the quantity of any remaining infectivity (I,v, $ID_{50}$s) in the pdFVIII product made from each pool.

The levels of reduction of vCJD agent achieved during manufacturing and processing varies among the pdF VIII products on the market. To our knowledge, the total level of reduction in vCJD agent achieved during manufacturing for any pdFVIII product on the market is not known. However, the levels of TSE clearance and reduction for some of the individual fractionation steps (or similar steps) used in the manufacture of some pdFVIII products is known. Based on TSE clearance studies in the published literature and manufacturers' data available to the FDA, FDA staff believe that the plasma-derived products currently on the market employ manufacturing processes that achieve a clearance of vCJD agent of 4 $\log_{10}$ or greater in the final pdFVIII product. The FDA model employed three stratifications of clearance:

- 2 – 3 $\log_{10}$
- 4 – 6 $\log_{10}$
- 7 – 9 $\log_{10}$

Each of these levels of clearance was modeled separately. Most of the results are presented for the 4-6 $\log_{10}$ reduction during manufacture processing in the risk characterization section (Section V.) of this risk assessment.

## Module 4: FVIII utilization and annual exposure

### *Background*

Patients with hemophilia A (HA) have an inherited – recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie *et al* 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a protein in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or a joint is common and can lead to severe disability or death without treatment. The complications of

HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Traditionally, HA patients were treated with factor concentrates only when bleeding occurred, which is called episodic treatment. Currently, the adoption of a preventative form of treatment or prophylaxis therapy is increasing. Prophylactic therapy refers to treatment regimens that seek to prevent bleeding events with regular infusions of clotting factor concentration. Prophylactic treatment greatly decreases the likelihood of adverse events such as joint hemorrhage, and effectively prevented the development of chronic joint arthropathy and disability, but requires higher use of FVIII product than episodic treatment.

Patients with vWD have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually necessitate treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

## IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII

The potential exposure of an individual HA or vWD patient or patient population with severe disease to the vCJD agent through use of pdFVIII was estimated in the model based on the:

- total quantity of pdFVIII used per year, and
- potential quantity of vCJD agent predicted in the pdFVIII product.

## IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients

The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen. Plasma-derived FVIII utilization and the size of each of the severe HA clinical treatment subpopulations were estimated using data from a Centers for Disease Control (CDC) sponsored study by 6 states by Hemophilia A patients from 1993-1998. This risk assessment provides outputs that estimate the annual exposure for several patient subpopulations with **Severe HA** disease for patients in the following clinical treatment groups:

- Prophylaxis – No inhibitor
- Prophylaxis - With inhibitor
- Prophylaxis - With inhibitor and immune tolerance
- Episodic – No inhibitor
- Episodic - With inhibitor

Because patients with severe HA are likely to use higher quantities of pdFVIII product we reasoned that they would be at potentially greater risk, than those with moderate or mild hemophilia, if the vCJD agent were present in US manufactured product.

A summary of the utilization data used for the model are provided in the table below. Additional technical details of the analysis of FVIII utilization for this population are described in section A-IV.G. of Appendix A.

**Table 4.8. Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution**

| | | Input distribution | | | |
|---|---|---|---|---|---|
| **Treatment Regimen** | **Inhibitor Status** | **n** | **(min, max)** | **Mean** | **95% CI** |
| *Prophylaxis* | No Inhibitor | 578 | (300, 1200000) | 157949 | (21242, 282316) |
| | With Inhibitor – No Immune Tolerance | 63 | (2000, 800000) | 190523 | (26956 , 447639) |
| | With Inhibitor – With Immune Tolerance | 62 | (100000, 2000000) | 558700 | ( 33235, 1592943) |
| *Episodic* | No Inhibitor | 946 | (0, 1000000) | 85270 | ( 4633, 244656) |
| | With Inhibitor | 151 | (2200, 1000000) | 160458 | (5314 , 488906 ) |

## IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients

The CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 did not include patients with vWD. We assumed that vWD patients with severe disease would largely use Humate P product only for factor replacement treatment. A search of records in the Hemophilia Surveillance System project data revealed a total of 58 records that indicated Humate P had been used, among which, 8 records indicates patients had developed inhibitor, which are considered uncommon among vWD patients and were excluded from analysis. Among the 58 records, 35 were from Adults (≥15 yrs of age) and 23 records were from young persons (<15 yrs of age). Records for each age group were further grouped by clinical treatment using either a prophylaxis or episodic treatment regimen. Data were initially analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate descriptive statistics and statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. The Generalized Beta distribution was identified as the best fit to the pdFVIII utilization data (as determined by using the software Best Fit (Palisade Corp, NY) and was used as the input distribution for pdFVIII usage by individual vWD patients in the model. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. Table 4.9. summarizes pdFVIII usage data from CDC sponsored study and the input distribution generated based on the data. FDA used data in the CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 to estimate FVIII utilization by all vWD patients. The data represent only a sample of all possible vWD patients with severe disease in the US. FDA estimated that there were approximately 250 patients in the US with Type 3 vWD. To calculate the total number of patients in each age group and treatment regimen group we adjusted the 58 patient population to equal a total of 250 patients by multiplying the patient population in each group by a factor of 4.3 (250/58 = ~4.3). The utilization data for patients in each treatment regimen in the sample population were used in the risk assessment model to generate outputs for the annual exposure to vCJD for all vWD for Adult (>15 yrs of age) and Young (≤15 yrs of age) persons in the US among clinical treatment groups of prophylaxis and episodic. The FVIII utilization data were used to calculate the potential vCJD risk for vWD patients; these results are shown in Tables 5.2A and 5.2B.

**Table 4.9. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution**

| | Input Distribution | | | |
|---|---|---|---|---|
| **Treatment Regimen** | **n** | **(min, max)** | **Mean** | **95% CI** |
| **Young** (≤15 yrs of age) | | | | |
| *Prophylaxis* | 9 | (9200, 504625) | 165713 | (9346, 479457) |
| *Episodic* | 14 | (1010, 41850) | 11045 | (1013, 37543) |
| **Adult** (>15 yrs of age) | | | | |
| *Prophylaxis* | 17 | (15000, 772800) | 186880 | (15570, 606670) |
| *Episodic* | 18 | (1000, 293800) | 86923 | (1362, 260660) |

# V. RISK CHARACTERIZATION

The risk characterization section of the risk assessment integrates information from the hazard identification, hazard characterization and the exposure assessment components to arrive at estimates of the risks posed by a hazard.

In this risk assessment data for hazard characterization are lacking, so we could not develop a human vCJD dose-response. The dose-response relationship provides information needed to use the exposure (dose) assessment results to estimate the probability of adverse responses including infection, illness or mortality – based on assessment of exposure (dose) to the hazard. Many TSE models and risk assessments, including our model, use the $ID_{50}$, or amount of material that leads to infection in 50% of the population, as a semi-quantitative estimate of the amount of TSE agent. The $ID_{50}$ has been derived from rodent animal models and may or may not approximate infection and occurrence of vCJD in humans. This lack of knowledge about the animal data and how they relate to actual human clinical vCJD outcomes adds considerable uncertainty to the risk estimates generated by the model. The FDA risk assessment interprets the $ID_{50}$ as representing a linear dose-response relationship or linear relationship between exposure and the probability of

infection. In such a case exposure to 1 $ID_{50}$ would suggest a 50% probability of infection, exposure to 0.1 $ID_{50}$ would suggest a 5% probability of infection, and so on.

The final results of this risk assessment provide estimates of potential annual exposure and annual vCJD infection risk for patients with severe HA and for patients with severe vWD for pdFVIII manufactured from plasma collected in the US. The risk was estimated by applying the linear $ID_{50}$ dose-response relationship, which provides a probability of vCJD infection in the two populations and various subpopulations within the two groups. Given the limited data available FDA believes that any extrapolation or interpretation has limited utility in actually estimating outcomes such as infection and illness. Therefore, any estimate of the risk based on estimates of exposure to the vCJD agent through use of pdFVIII will be imprecise and extremely uncertain.

## V.A. THE MODEL

This risk assessment and simulation model links the available scientific and epidemiological data together to mathematically approximate the processes (predicted presence of vCJD in UK population, manufacturing, reduction of vCJD agent, and patient utilization) leading to potential exposure of US patients to vCJD agent present in US-manufactured pdFVIII. A summary of the variables, parameters and equations used in the model were described in Section III. Exposure Assessment and a summary of the variables and equations, data, and assumptions used in the model are provided in Appendix A. The model was run using @Risk software package (Palisades Corp, NY) to conduct the Monte Carlo analysis. Simulations of 10,000 iterations were run.

The risk assessment uses Monte Carlo simulation to randomly draw values from probability input distributions (which are statistical representations of input data) once per iteration; thousands of iterations are used to generate the model outputs as risk estimates. This simulation method is often used in situations when a model is complex, non-linear, or involves several uncertain parameters. The output generated is usually an aggregate distribution whose shape can be summarized using measures of central tendency (mean, median, mode) or with boundaries such as the 95% confidence interval (CI), the $5^{th}$ and $95^{th}$ percentiles (representing the 90% CI) or the range, bounded by the minimum and maximum values generated as part of the output. The strength of Monte Carlo analysis is that it generates resulting risk estimates as statistical distributions, which reflect the underlying uncertainty and variability of the original input data and parameters.

## V. B. Model results: Estimated annual potential exposure to vCJD i.v. $ID_{50}$ and potential vCJD risk through human pdFVIII used to treat severe HA

Individuals with HA vary in their degree of FVIII deficiency. Although the clinical spectrum generally can range from severe, to moderate, and to mild disease, this

assessment specifically addresses potential vCJD exposure and risk for persons with severe HA. Among an estimated 14,000 HA population in the United States, approximately 50% have severe disease and 25% of all HA patients use human pdFVIII products. FDA estimated that there are a total of approximately 1,800 HA patients **(Tables 5.1A. and 5.1B.)** with severe disease in the US that use human pdFVIII products. Although the estimated risk is very low, it is possible that some patients using human pdFVIII may potentially be exposed to vCJD agent if present in US manufactured product.

*Estimation of PdFVIII product utilization by patients with severe HA.* FDA obtained data on human plasma-derived FVIII utilization from the Centers for Disease Control (CDC). Data in the study were collected as part of a collaborative effort between CDC and six states during the time period 1993 – 1998. A summary of study results for New York State are described in Linden, et al. (2003). The comprehensive study collected standardized patient demographic, clinical, treatment and outcome data. Patient medical records were obtained from treatment sites including: hemophilia treatment centers (HTCs), hospitals, clinics, physician's offices, home-care agencies, nursing homes, prison infirmaries, and dispensers of factor concentrates. The data abstracted from medical records tabulated all factor concentrate utilization prescribed by quantity, type, purpose (e.g., prophylaxis, treatment of acute bleeds, or immune tolerance therapy) and total quantity used per calendar year.

The data on quantity of pdFVIII product utilized annually were used to develop statistical distributions of product usage for patients by treatment group. The mean quantities of products utilized by HA patients on different treatment regimens are shown in **Table 5.1A. and 5.1B**. Approximately 1,100 records for patients utilizing pdFVIII were analyzed in this study. The percentage of each patient subpopulation in proportion to the total HA population in the CDC-Six State study was used to extrapolate the estimated number of total individuals in each patient subpopulation. From the study results, we estimated that there are a total of approximately 1,800 persons with severe HA in the US who use pdFVIII.

Results from the risk assessment model for patients with severe HA who are treated with pdFVIII product with a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in **Tables 5.1A. and 5.1B.** Generally results are expressed for patients in several different HA clinical treatment groups including:

- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

*Potential exposure of severe HA patients to vCJD agent: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (based on Clarke and Ghani, 2005).* The model estimates that severe HA patients treated using a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) has the highest pdFVIII usage of the groups we examined

and potentially face the highest risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year and are potentially exposed to an average of $1.57 \times 10^{-6}$ i.v. $ID_{50}$ per person per year; representing an average potential vCJD risk of 1 in 1.3 million per person per year. If all of the assumptions in the model are correct at this lower estimated prevalence, this risk may yield 1 vCJD infection in an average of approximately 21,000 years of treatment among severe HA patients who are in a prophylaxis treatment regimen with inhibitor and immune tolerance. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) in **Table 5.1A.** are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicts that 0.027% of the time the exposure to vCJD agent may be greater than zero, and there is a possible but low risk of vCJD infection.

The risk for the entire population is calculated by summing the cumulative risk potential of vCJD exposure and risk **(Table 5.1B.)**. Using the lower prevalence estimate, the model predicts that the approximately 1,800 severe HA patient population in the US uses a total of approximately 243 million IU pdFVIII and is exposed to an average of $6.50 \times 10^{-4}$ i.v. $ID_{50}$. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential population-based vCJD risk of 1 in 3,077. At this expected level of risk, 1 vCJD infection would be predicted to occur in 3,077 years of treatment for the entire population of 1800 severe HA patients that use pdFVIII.

*Potential exposure of severe HA patients to vCJD agent: Results based on higher surveillance prevalence estimate of 1 in 4,225 (Hilton, et al 2004).* The model estimates that severe HA patients in a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) potentially face the highest expected risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year, and are potentially exposed to an average of $1.30 \times 10^{-4}$ i.v. $ID_{50}$ per person per year, using the higher prevalence estimate. This represents an average potential vCJD risk of 1 in 15,000 per person per year for the treatment group. If all of the assumptions used in the model are correct and considering the total number of 62 patients in this category (or population-based risk), this expected risk would yield 1 vCJD infection in 240 years of treatment among the patients under this category.

The risk for the entire severe HA population is calculated by summing the cumulative risk potential of vCJD exposure and risk from all individual patients under five categories (prophylaxis with no inhibitor, prophylaxis with inhibitor, prophylaxis with inhibitor and immune tolerance, episodic with no inhibitor and episodic with inhibitor) **(Table 5.1B.)**. Using the higher surveillance estimate, the model predicts that the approximate total of 1,800 severe HA patient population in the US uses a total of approximately 243 million IU

pdFVIII, and is exposed to an average of $5.67 \times 10^{-2}$ i.v. $ID_{50}$ per year. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential population-based vCJD risk of 1 in 35, i.e., 1 vCJD infection would be predicted to occur in 35 years of treatment in this 1800 severe HA patient population.

**Table 5.1A. Model Results for All HA Patients who use a Hypothetical Factor VIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent:** ***Predicted Annual per Person Exposure to vCJD i.v. $ID_{50}$ and Mean Potential per Person Annual vCJD Risk:***

- For patients with SEVERE disease, and
- Two different UK vCJD prevalence estimates.

| | | | | **4 - 6 $Log_{10}$ Reduction** | | | |
|---|---|---|---|---|---|---|---|
| | | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton *et al* (2004) | |
| **Treatment Regimen** | **Inhibitor Status** | **Est. Total Number patients in US** | **Mean quantity FVIII used per person per year ($5^{th}$ - $95^{th}$ perc)** | **Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c]** | **Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c]** | **Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c]** | **Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c]** |
| ***Prophylaxis*** | No Inhibitor | **578** | **157949 IU[d]** (21242 , 382316 ) | **$4.99 \times 10^{-7}$** (0-0) [e] | 1 in 4.0 million (0-0) [e] | **$3.67 \times 10^{-5}$** (0 - $1.72 \times 10^{-4}$) | 1 in 54,000 (0 - 1 in 12,000) |
| | With Inhibitor – No Immune Tolerance | **63** | **190523 IU[d]** (26956, 447639) | **$4.21 \times 10^{-7}$** (0-0) [e] | 1 in 4.8 million (0-0) [e] | **$4.86 \times 10^{-5}$** (0 - $2.17 \times 10^{-4}$) | 1 in 41,000 (0 - 1 in 9,000) |
| | With Inhibitor – With Immune Tolerance | **62** | **558700 IU[d]** ( 33235, 1592943) | **$1.57 \times 10^{-6}$** (0-0) [e] | 1 in 1.3 million (0-0) [e] | **$1.30 \times 10^{-4}$** (0 - $5.39 \times 10^{-4}$ ) | 1 in 15,000 (0 - 1 in 3,700 ) |
| ***Episodic*** | No Inhibitor | **946** | **85270 IU[d]** ( 4633, 244656) | **$2.12 \times 10^{-7}$** (0-0) | 1 in 9.4 million (0-0) [e] | **$1.91 \times 10^{-5}$** (0 - $8.50 \times 10^{-5}$) | 1 in 105,000 (0 - 1 in 24,000 ) |
| | With Inhibitor | **151** | **160458 IU[d]** (5314, 488906 ) | **$2.49 \times 10^{-7}$** (0-0) [e] | 1 in 8.0 million (0-0) [e] | **$4.19 \times 10^{-5}$** (0 - $1.67 \times 10^{-4}$) | 1 in 48,000 (0 - 1 in 12,000 ) |

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## Table 5.1B. Model Results for Total Population-based Exposure and Potential vCJD Risk for All Hemophilia A patients who use a Hypothetical pdFVIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent:

Predicted annual per person exposure to vCJD i.v. $ID_{50}$ and mean potential per person annual vCJD risk:

- For patients with SEVERE disease, and
- Two different UK vCJD prevalence estimates.

| | | | 4 - 6 $Log_{10}$ Reduction | | | |
|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | |
| | Est. Total Number severe HA patients in US | Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] |
| Mean total annual exposure and population risk | 1,800 | 243 million IU[d] | $6.50 \times 10^{-4}$ (0-0)[e] | 1 in 3,077 years (0-0)[e] | $5.67 \times 10^{-2}$ (0 - $2.52 \times 10^{-1}$) | 1 in 35 years (0 - 1 in 8 ) |

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## V. C. Model results: Estimated annual potential exposure to i.v. $ID_{50}$ vCJD agent and potential vCJD risk through human pdFVIII used to treat severe von Willebrand disease (vWD)

Individuals with von Willebrand disease (vWD) vary in severity of disease, those with Type 3 disease have severe disease; this assessment specifically addresses potential vCJD exposure and risk for persons with severe vWD. FDA estimates that approximately 250 vWD patients have severe vWD disease in the United States and use human plasma-derived FVIII products to control their disease (Tables 5.2A. and 5.2 B.) The FDA model suggests that it is possible that some of these vWD patients using human pdFVIII may potentially be exposed to vCJD agent if present in US manufactured product. Results from the risk assessment model for patients with vWD and treated with pdFVIII product with a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in Tables 5.2A. and 5.2 B. Generally results are expressed for patients with von Willebrand disease (vWD) clinical treatment groups of either Prophylaxis or Episodic treatment.

**Table 5.2A. Results von Willebrand Disease (vWD) patients[1] with Severe Disease: Predicted Potential Annual Exposure to vCJD i.v. $ID_{50}$ and vCJD Risk:**

- Assuming a processing reduction of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

| YOUNG vWD (≤ 15 yrs of age) | | | | | | |
|---|---|---|---|---|---|---|
| | | | 4 - 6 $\text{Log}_{10}$ Reduction | | | |
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004) | |
| | Est. Total Number patients in US | Mean quantity product used per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] |
| *Prophylaxis* | 39 | 165,713 IU[d] (9876, 454306) | $4.30\times10^{-7}$ ($0$ - $0$)[e] | 1 in 4.7 million ($0$ - $0$)[e] | $3.81\times10^{-5}$ ($0$ - $1.54\times10^{-4}$) | 1 in 52,000 ($0$ - 1 in 13,000) |
| *Episodic* | 60 | 11,045 IU[d] (1025, 34352) | $4.14\times10^{-8}$ ($0$ - $0$)[e] | 1 in 48 million ($0$ - $0$)[e] | $2.06\times10^{-6}$ ($0$ - $6.83\times10^{-6}$) | 1 in 971,000 ($0$ - 1 in 293,000) |
| ADULT vWD (> 15 yrs of age) | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Prophylaxis*** | **73** | **186,880 IU[d]** (16910, 539877) | **$4.89\times10^{-7}$** (0 - 0)[e] | 1 In 4.1 million (0 - 0)[e] | **$4.32\times10^{-5}$** (0 - $1.82\times10^{-4}$) | 1 in 46,300 (0 - 1 in 11,000) |
| ***Episodic*** | **78** | **86,923 IU[d]** (2182, 240338) | **$1.99\times10^{-7}$** (0 - 0)[e] | 1 In 10 million (0 - 0)[e] | **$1.90\times10^{-5}$** (0 - $8.43\times10^{-5}$) | 1 in 1 million (0 - 1 in 24,000) |

[1]Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity i.v. $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents International units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

*Estimation of Factor VIII product utilization by patients with severe von Willebrand disease.* FDA obtained data on pdFVIII utilization, presumably used in the treatment of severe von Willebrand disease, from the Centers for Disease Control (CDC). Details of the CDC – Six state collaborative study are described in the section above (section IV.G.2) on FVIII utilization. Annual usage of product by vWD patients was estimated based on an assumption that this patient class largely uses Humate P. Therefore, only records for patients utilizing Humate P were extracted from the CDC - Six state study conducted from 1993 – 1998 and used to develop statistical distributions of product usage for young vWD (≤15 yrs old) patients and adult vWD (> 15 yrs old) patients. The mean quantity of product utilized per year per patient group is shown in **Table 5.2A.** and **Table 5.2B.**

**Table 5.2B. Von Willebrand Disease (vWD) Patients[1] with Severe Disease: Predicted Total Population-based Exposure to vCJD i.v. $ID_{50}$ and Potential vCJD Risk:**

- Assuming a processing reduction of 4-6 log $_{10}$ , and
- Two different UK vCJD prevalence estimates.

| | | 4 - 6 $Log_{10}$ Reduction | | | |
|---|---|---|---|---|---|
| | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton *et al* (2004) | |
| **Est. Total Number severe vWD patients in US** | **Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c]** | **Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c]** | **Mean population – based potential vCJD risk[b] (5th - 95th perc)[c]** | **Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c]** | **Mean population – based potential vCJD risk[b] (5th - 95th perc)[c]** |

| | | | | | | |
|---|---|---|---|---|---|---|
| ***Mean total annual exposure and population risk*** | **250** | **29.9 million IU[d]** (3013, 311745) | **$7.05 \times 10^{-5}$** (0 - 0)[e] | **1 in 28,450 years** (0 - 0)[e] | **$4.91 \times 10^{-3}$** (0 - $2.59 \times 10^{-2}$) | **1 in 405 years** (0 - 1 in 76) |

[1]Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[a]iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b]Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity i.v. $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c]The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d]IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

*Potential exposure of severe von Willebrand disease patients to vCJD agent: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani, 2005).* Adult vWD (>15yrs of age) patients with severe disease on prophylaxis consumed the largest quantities of pdFVIII product annually and may potentially be at greater vCJD risk. Using the lower epidemiological model prevalence estimate, analysis of pdFVIII utilization data indicated that 73 Adult vWD patients on prophylaxis treatment regimen used an average of 186,880 IU and are potentially exposed to an average of $4.89 \times 10^{-7}$ i.v. $ID_{50}$ per person per year, and representing an average potential vCJD risk of 1 in 4.1 million per person per year **(Table 5.2A.)**. At this level of risk, only 1 vCJD infection would be predicted to occur in an average of approximately 56,000 years. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) in **Table 5.2A.** are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicts that 0.027% of the time the exposure to vCJD agent may be greater than zero, and there is a possible but low risk of vCJD infection.

Totaling the model results reveals that the approximately 250 severe vWD patients in the US used a total of 29.9 million IU, and are potentially exposed to an average total of $7.05 \times 10^{-5}$ i.v. $ID_{50}$ per year. This represents an average potential vCJD risk of 1 in 28,450 **(Table 5.2B.)** or (as predicted by the model) roughly equal to one vCJD infection observed over a time span of approximately 28,450 years in the population of 250 severe vWD patients.

*Potential exposure of severe von Willebrand disease patients to vCJD agent: Results based on higher prevalence estimate of 1 in 4,225 (Hilton et al 2004).* At the higher surveillance prevalence estimate, among the vWD patient populations examined by the model, results **(Table 5.2A.)** indicated that adult vWD (>15yrs of age) patients with severe disease on prophylaxis used the largest quantities of pdFVIII product annually and may potentially be at greater vCJD risk. Analysis of pdFVIII utilization data indicated that 73 Adult vWD patients on prophylaxis treatment regimen used an average of 186,880 IU per person per year and are potentially exposed to an average of $4.32 \times 10^{-5}$ i.v. $ID_{50}$ per person per year,

representing an average potential vCJD risk of 1 in 46,300 per person per year **(Table 5.2A.)**. At this level of risk, only 1 vCJD infection would be predicted to occur in an average of approximately 630 years for the population of 73 Adult vWD patients on prophylaxis treatment regimen.

The potential risk of vCJD infection for the entire population was calculated using the higher surveillance prevalence estimate. The model results shows that the approximately 250 severe vWD patients in the US used a total of 29.9 million IU **(Table 5.2B.)**, and are potentially exposed to an average total of $4.91 \times 10^{-3}$ i.v. $ID_{50}$ per year. This represents an average potential vCJD risk of 1 in 405, i.e., of one vCJD infection observed over a time span of 405 years for the population of 250 severe vWD patients in the U.S.

*Range of Predicted annual mean potential per HA patient vCJD risk for pdFVIII (Table 6)*
The FDA risk assessment for potential vCJD infection risk for US manufactured pdFVIII generates results for several scenarios that reflect two key factors that greatly influence the final risk estimates including: (1) Reduction in vCJD agent in pdFVIII product during manufacture, and (2) UK vCJD prevalence estimate. As indicated earlier, the model used two widely different prevalence estimates, one lower prevalence estimate based on epidemiological modeling of predicted vCJD cases in the UK (Clarke and Ghani, 2005) of approximately 1.8 in 1 million and one higher prevalence estimate based on surveillance data of UK patient tissue samples (Hilton et al 2004) of 1 in 4,225. The use of these two estimates gives rise to a difference in results generated by the model that vary by an average of approximately 130 fold.

The model evaluated three separate categories of reduction in infectivity including 2-3 $\log_{10}$, 4-6 $\log_{10}$, and 7-9 $\log_{10}$. These three hypothetical categories were chosen to span the possible range of reduction of vCJD agent for pdFVIII products. **Table 5.3A. and 5.3B.** displays model results for a lower prevalence estimate and a higher prevalence estimate at all three levels of reduction. It should be noted that the mean difference between the lowest range of 2-3 $\log_{10}$ and the highest range of 7-9 $\log_{10}$ is nearly 1 million fold (6 $\log_{10}$). These two largest contributors to the final risk estimate also contribute to the greatest uncertainty in the model. Results from the model shown in **Tables 5.3A. and 5.3B.** indicate that there is a difference of approximately 20 to 55 million fold between the lowest and highest risk estimates of each patient group.

**Table 5.3A. Range of *Predicted Annual Mean Potential per HA Patient vCJD risk for pdFVIII* –** at *three levels of clearance: 7-9 $log_{10}$, 4-6 $log_{10}$, and 2-3 $log_{10}$ and at a higher Prevalence and Lower Prevalence estimates and at .*

| | | | | 7 - 9 $Log_{10}$ Reduction | | 4 - 6 $Log_{10}$ Reduction | | 2 - 3 $Log_{10}$ Reduction | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on** Clark and Ghani (2005) | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton *et al* (2004) | **Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on** Clark and Ghani (2005) | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton *et al* (2004) | **Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on** Clark and Ghani (2005) | **Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225** by Hilton *et al* (2004) |
| **Treatment Regimen** | **Inhibitor Status** | **Est. Total Number patients in US** | **Mean quantity product used per person per year ($5^{th}$ - $95^{th}$)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** | **Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b]** |
| ***Prophylaxis*** | No Inhibitor | **578** | **157949 IU** (21242 , 382316 ) | 1 in 4.1 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 4 million (0-0)[c] | 1 in 54,000 (0- 1 in 12,000) | 1 in 15,000 (0-0)[c] | 1 in 82 (0 - 1 in 17) |
| | With Inhibitor – No Immune Tolerance | **63** | **190523 IU** (26956 , 447639) | 1 in 3.5 billion (0-0)[c] | 1 in 40 million (0 - 1 in 8.8 million) | 1 in 4.8 million (0-0)[c] | 1 in 41,000 (0- 1 in9,000) | 1 in 12,000 (0-0)[c] | 1 in 65 (0 - 1 in 13 ) |
| | With Inhibitor – With Immune Tolerance | **62** | **558700 IU** ( 33235, 1592943) | 1 in 551 million (0-0)[c] | 1 in 15 million (0 - 1 in 3.4 million) | 1 in 1.3 million (0-0)[c] | 1 in 15,000 (0- 1 in3,700) | 1 in 2,700 (0-0)[c] | 1 in 24 (0 - 1 in 3 ) |
| ***Episodic*** | No Inhibitor | **946** | **85270 IU** ( 4833, 244656) | 1 in 3.2 billion (0-0)[c] | 1 in 100 million (0 - 1 in 24 million) | 1 in 9.4 million (0-0)[c] | 1 in 105,000 (0- 1 in 24,000) | 1 in 21,500 (0-0)[c] | 1 in 159 (0 - 1 in 34 ) |
| | With Inhibitor | **151** | **160458 IU** (5314 , 488906 ) | 1 in 4 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 8 million (0-0)[c] | 1 in 23,000 (0- 1 in 12,000) | 1 in 23,000 (0-0)[c] | 1 in 73 (0 - 1 in 16) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range constituting the 90% confidence interval. Accordingly, the mean risk estimates from the model should fall within this defined interval at least 90% of the time.

[c] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

This range or difference in the estimates of about 20 -55 million fold is reflected in the higher and lower prevalence results generated by the model shown in **Table 5.3A.** for each HA patient treatment group with severe disease. On closer inspection of the results in **Table 5.3A.** for patients with the most intensive pdFVIII product use, that is, the 62 patients on prophylaxis-with inhibitor and with immune tolerance, the effect of clearance on mean potential vCJD risk across the three ranges of clearance can be seen. At the low end of risk, the mean potential vCJD risk per patient per year risk (at 7-9 $log_{10}$ and the lower prevalence estimate) is 1 in 551 million. Conversely, the highest risk for this patient group is seen at the 2-3 $log_{10}$ clearance level and the higher prevalence estimate and is estimated by the model to be an average of 1 in 24. For patients on episodic treatment with no inhibitor who have a less intensive annual use of product, the model predicts the lowest risk (at 7-9 $log_{10}$ and the lower prevalence estimate) to be 1 in 3.2 billion. The model predicts the highest risk for this group of patients, if they used pdFVIII product with a 2-3 $log_{10}$ clearance level and the higher prevalence estimate, would be a mean potential per patient risk of 1 in 159.

**Table 5.3B. Range of Total Population-based Exposure and Potential vCJD Risk from Model** Predicted HA population with severe disease annual vCJD Exposure and Risk associated with use of plasma-derived Factor VIII:

- Lower Prevalence assumptions of Prevalence of 1.8 in 1,000,000 and 7-9 $log_{10}$ reduction, and
- Higher Prevalence assumptions of Prevalence of 1 in 4,225 and 2-3 $log_{10}$ reduction.

| | | | 7 - 9 Log$_{10}$ Reduction | | 4 - 6 Log$_{10}$ Reduction | | 2 - 3 Log$_{10}$ Reduction | |
|---|---|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton et al (2004) | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) |
| | Est. Total Number severe vWD patients in US | Mean Total quantity FVIII used by all patients per year (5$^{th}$ - 95$^{th}$ perc)[b] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c])[c] | Mean population – based potential vCJD risk[a] (5$^{th}$ - 95$^{th}$ perc)[c] |
| ***Mean total annual exposure and population risk*** | **1,800** | **243 million IU** | **1 vCJD infection in 1.6 million years** (0-0)[c] | **1 vCJD infection in 35,000 years** (0 -1 in 9,000) | **1 vCJD infection in 3,100 years** (0 -0)[c] | **1 vCJD infection in 40 years** (0 -1 in 10) | **1 vCJD infection in 8 years** (0 -1 in 2) | **~13 vCJD Infections per year** (0- 54 vCJD infections) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The 5$^{th}$- 95$^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[c] For a 5$^{th}$ and 95$^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

The results from the risk assessment model shown in **Table 5.3A.** show a wide range of difference in the predicted risk and displays the range in our uncertainty and knowledge in

predicting the potential vCJD infection risk for HA patients who use US manufactured human pdFVIII. However, as further scientific information and data become available in the future, the uncertainty in the model may decrease and the estimates of vCJD risk for recipients of pdFVIII may become more precise.

Evaluating the total vCJD infection risk for the severe HA population of 1,800 by summing the total annual exposure (at the higher vCJD Infection prevalence estimated), the model predicts that the population would use a total average of approximately 243 million IU FVIII. If the patient population used product that attained a clearance of 7-9 $\log_{10}$ and assuming the lower prevalence the model predicts that for the total patient population the mean total annual risk would be 1 case in 1.6 million years representing a negligible vCJD risk that would likely not give rise to new cases of the disease. At the other end of the spectrum at the 2-3 $\log_{10}$ clearance level and the higher prevalence the model predicts a mean of approximately 13 vCJD infections per year **(Table 5.3.B.)** for the patient population.

## V. D. Sensitivity analysis

Sensitivity analysis is used to identify the input parameter or parameters that have the greatest impact on the risk estimates generated by the model and are done by varying the values of key input parameters and evaluating the effect on the final risk estimate. Our goal in doing these analyses was to identify the key input parameters that have the greatest influence on annual exposure to the vCJD agent. The model was examined and candidate variables for the sensitivity analysis were chosen from the model that exhibited the largest potential for variability and/or uncertainty and those values are listed in Table 5-7. Importance analysis is a type of sensitivity analysis. Our importance analysis used two values, one at the minimum or 5$^{th}$ percentile value and one at the maximum or 95$^{th}$ percentile value to provide a reasonable estimate of impact across the range tested. The results from the importance analysis are displayed as tornado plots (**Figures 2.A., 2.B. and 2.C.**), which graphically shows the relative influence of each input parameter evaluated on the final model estimates. The most influential factors are displayed at the top of the plot and those that are least influential or those with negative influence on the risk are at the bottom of the plot.

For the FVIII risk assessment the output being monitored in the sensitivity and importance analyses was annual exposure ($I_{yr}$) to vCJD agent quantified in i.v.$ID_{50}$ units. The sensitivity and importance analysis were conducted using the HA patient population on prophylaxis treatment regimens with inhibitor and being treated for immune tolerance as the example population used to do the analyses. This population displayed the largest mean usage and the widest range in product utilization. We assumed that the sensitivity and importance analysis results are representative of all the HA and vWD patient populations included in our study since all of the populations were assumed to differ only by the total average quantity of pdFVIII utilized per year.

The importance analysis was performed for each variable by doing two sets of simulations, each with 5,000 iterations. For each set of simulations the value of one testing variable was set at the minimum or 5$^{th}$ percentile value for the input distribution and the simulation run; for the second run the variable was set at the maximum or 95$^{th}$ percentile value and the simulation run. The importance analysis was run separately each time using one of the three surveillance estimate

ranges. The first analysis used a range of 0.7 to 700 per million, which encompasses the entire range for both the HIGH and LOW prevalence estimates. The second analyses used the higher vCJD Infection prevalence estimate of 1 in 4,225 (or 237 per million) derived from a tissue surveillance study (Hilton et al 2004). This prevalence was based on the variable ($P_{vCJD\text{-}Surv}$) in the model that used data from a tissue surveillance study. To do the sensitivity analysis we used a 5$^{th}$ percentile value of 49 per million and a 95$^{th}$ percentile value of 692 per million. The third set of analyses used the lower vCJD Case prevalence estimate of ~1.8 per million based on epidemiological modeling from actual vCJD occurrence conducted by Clarke and Ghani (2005). This prevalence was ($P_{vCJD\text{-}Epi}$) based on epidemiologic modeling and to do the sensitivity analysis we used a 5$^{th}$ percentile value of 0.7 per million and a 95$^{th}$ percentile value of 4 per million. The results of all simulations and the ranking of input parameters by their importance is represented graphically using a tornado plot shown in **Figures 2.A. , 2.B. and 2.C.** The tornado plot displays the correlations between key inputs in the model and the model output of exposure. A tornado plot prioritizes the various input factors with the most influential factors at the top and those that are least influential or those with negative influence on the risk are at the bottom of the plot.

**Table 5.4. Input Variables included in Importance Analysis**

| Description of variables | Name of input variable | Importance analysis values |
|---|---|---|
| Entire range of estimated vCJD prevalence in UK (cases/million) | $Prev_{vCJD\text{-}UK}$ | Minimum: 0.7<br>Maximum: 700 |
| High prevalence estimate of vCJD in UK (cases/million) | $Prev_{vCJD\text{-}UK(Surveillance)}$ | 5$^{th}$ perc: 49<br>95$^{th}$ perc: 692 |
| Low vCJD prevalence in UK (cases/million) | $Prev_{vCJD\text{-}UK\ (Epi\ model)}$ | 5$^{th}$ perc: 0.7<br>95$^{th}$ perc: 4.0 |
| Efficiency of donor deferral policy | $Eff_{Def}$ | Minimum: 85%<br>Maximum: 99% |
| Efficiency of i.c. versus i.v. route | $A_{ic\text{-}iv}$ | Minimum: 0.1<br>Maximum: 1 |
| Number of donors per plasma pool | $DR_{Pool}$ | Minimum: 6500<br>Maximum: 360000 |
| Quantity of i.c. infectivity in infected human blood | $I_{bl}$ | 5$^{th}$ perc: 2<br>95$^{th}$ perc: 30 |
| Manufacturing yield of FVIII (IU/L plasma) | $Y_{VIII}$ | Minimum: 120<br>Maximum: 250 |
| Log Manufacture Reduction of vCJD agent | $R_{Log}$ | Minimum: 2<br>Maximum: 9 |
| FVIII used per year (IU/year) | $IU_{yr}$ | 5$^{th}$ perc: 10000<br>95$^{th}$ perc: 4000000 |

Sensitivity analysis is used to study the quantitative relationship between the input variables and risk output. Same as in importance analysis, output to be monitored in sensitivity analysis is annual exposure ($I_{yr}$) to vCJD of young HA patients under prophylaxis treatment with inhibitor and immune tolerance treatment. Sensitivity analysis for an input variable consists of multiple simulations. In each simulation the testing input variable is fixed at one value within the input range. Results of sensitivity analysis are presented only for the most important input variables, which were identified by the ranking provided by the importance analysis.

**Fig 2. A. Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using prevalence estimate encompassing the range of values for both high and low prevalence from 0.7 to 700 vCJD cases per million UK population.** Tornado chart showing impact of input variables on estimated annual exposure of severe HA patient with prophylaxis, inhibitor and immune tolerance treatment

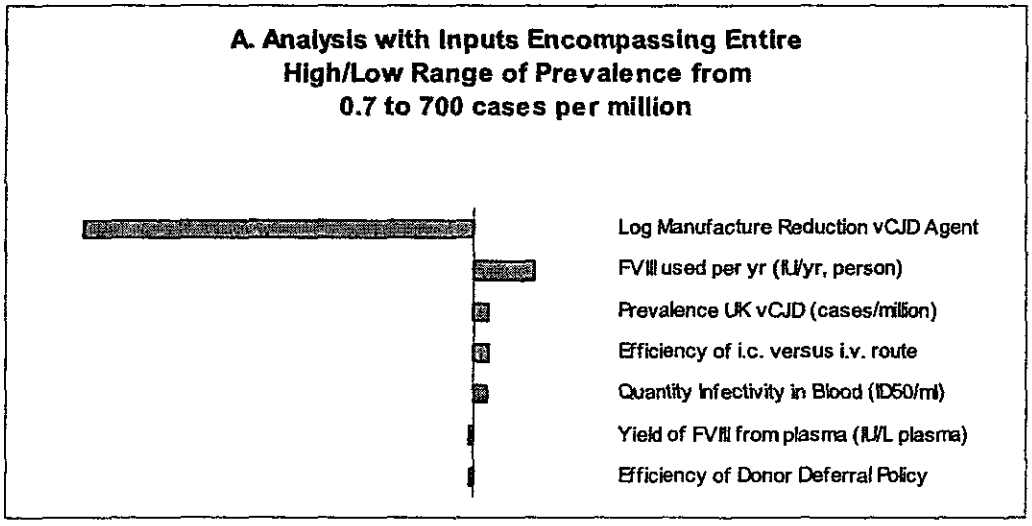



**Fig 2. B. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Tissue Surveillance-based (HIGH) prevalence estimate.** Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.

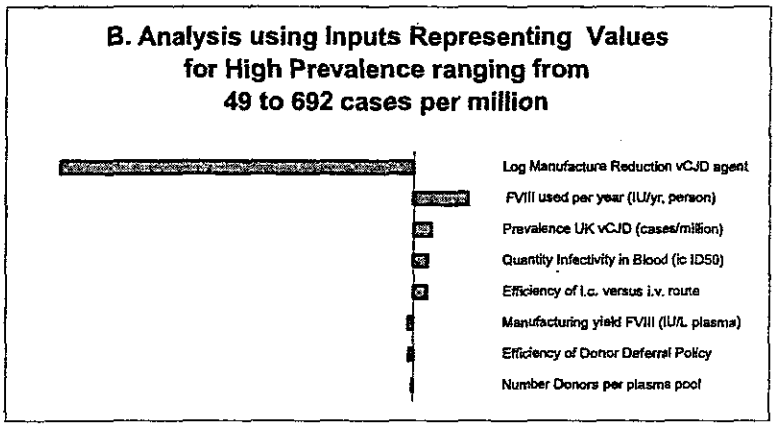
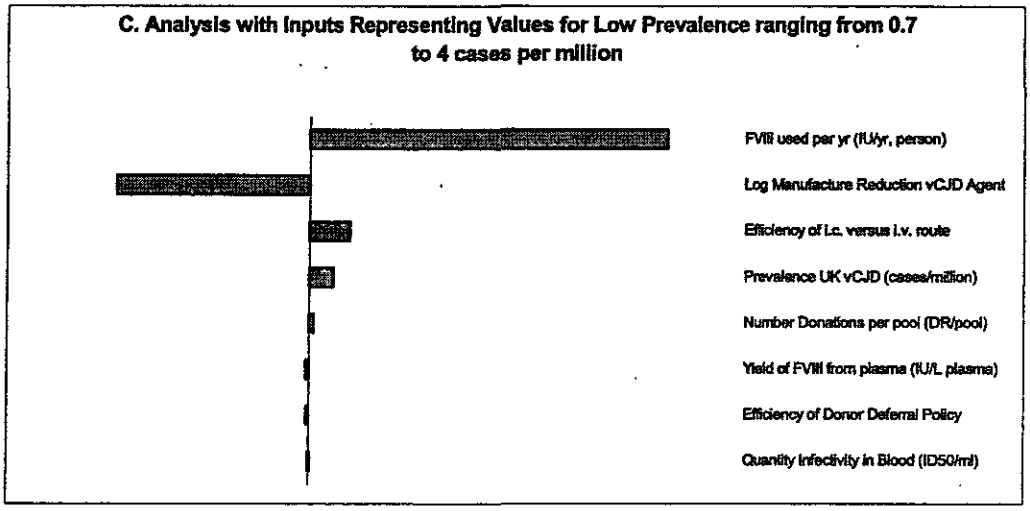


**Fig 2. C. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Epi Modeling-based (LOW) prevalence estimate.** Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.




Some input variables are used multiple times in the original model, for instance each type of plasma pool (Source or recovered) was modeled on an individual basis. Other examples are pool size ($DR_{pool\text{-}S}$ and $DR_{pool\text{-}R}$), yield ($Y_{FVIII}$), quantity of i.c. infectivity in the infected human blood ($I_{bl}$) and the reduction of infectivity during manufacturing ($R_{Log}$). In importance analysis and sensitivity analysis, when these input variables are tested, we assumed that there was no difference among the pools. When evaluating the impact of a specific variable all other values are held constant during the simulation. When simulating parameters with multiple values (e.g., size of recovered plasma pools) all values are the same for the simulation. The magnitude of changes in risk output associated with changes of input variables are graphed in the tornado chart, which represents the relative ranking of the input variables by their impacts on the risk outcome. The importance analysis was conducted for three possible ranges of UK vCJD prevalence: one set of analysis for tonsil survey based estimate, one set for epidemiology model-based estimate and another set for the two prevalence estimates combined.

The order of the influence of the specific input factors varies slightly when the importance analysis is conducted using the three difference prevalence estimates. When a higher prevalence estimate was used (either the combined prevalence (0.7 to 700 per million) the tornado plots in **Figures 2.A. and 2.B.** both show that clearance or Log reduction of the vCJD agent ($R_{Log}$) during the manufacturing process is the dominant factor that influences the annual exposure or risk for a pdFVIII recipient. The importance analysis suggests that changes in the input values for prevalence used in the analysis can cause some visible changes in the rank order of the influence of the various input factors. A change in the rank order of model factors is seen when the lower prevalence estimate of 0.7 to 4 per million is used (**Figure 2. C.**). The dominant factor potentially driving risk then becomes the quantity of pdFVIII used by a patient.

In our importance analysis the five variables that had significant influence on the output of the model were clearance of i.v. $ID_{50}$ from pdFVIII products, pdFVIII use (IU/yr), UK vCJD prevalence, adjustment for the efficiency of transmission via the i.c. route vs. the i.v. route, and the quantity of i.v.$ID_{50}$ in blood. Changes in prevalence did cause the variable parameters to reassort and change rank when the different prevalence estimates were used. Overall, however, they were somewhat similar in asserting their influence on the estimated risk outcome(s), but had significantly less influence when compared to that of reduction of infectivity during processing and manufacture. Although these types of sensitivity analysis and tornado plots are often used to identify influential factors of risk, their use has some limitations. Factors are examined singly or in isolation so interaction among various factors that may influence the risk estimate are not addressed.

## General comments on model outputs

The risk estimations in this section of the risk assessment are predicated on the assumption that there is homogeneous mixing and dispersion of vials from all pools among all donors. In reality, vials may not be dispensed homogeneously and it is likely that patients draw from only one or a few manufactured lots of pdFVIII product in a given year. FDA did not have data to model this non-homogeneous dispensing of pdFVIII but the model can be used to estimate the average maximal level of i.v. $ID_{50}$ exposure if on a very rare chance all vials used by a patient in a given year happened to contain vCJD agent.

## V. E. Uncertainty and Data Gaps

Uncertainty arises from the absence of information or availability of limited information. In our probabilistic model statistical distributions are used, where possible, to represent the uncertainty of much of the information used in the model. There are uncertainties in the information and the model that we were unable to quantify and that are not represented in the final risk estimates. Some of the difficult to quantify uncertainties are associated with the extrapolation of a human dose-response relationship based on animal data, an assumed linear dose response with no uncertainty or variability bounds, and assumption of infectivity in the last 50% of the incubation period. We express the uncertainty of the final risk estimates generated from the model using a mathematical mean (average) of exposure in $ID_{50}$ units and the $5^{th}$ and $95^{th}$ percentiles, which represent the 90% confidence interval for each estimate. The uncertainty for the risk estimates generated by this FVIII risk assessment model is significant and decision makers should use the results with caution. Similarly, patients and physicians should understand that the uncertainties are too great at this time to determine the presence, absence or degree of actual risk. In the future, additional research and information may be substituted for assumptions or used to improve estimates for the individual parameters and ultimately improve the precision of the final risk estimates generated by the model.

Even considering the associated uncertainty of estimated risks, risk assessment provides an estimate of risk based on the current and known information. It is still a useful tool that can inform the science-based decision making process. It can identify data gaps and research priorities where additional research and information would have the greatest impact on enhancing the final risk estimates. The sensitivity analysis results in Section IV.C. indicated that the risk

assessment results are highly dependent upon log reduction of vCJD agent ($R_{Log}$) during the manufacturing process. The modeled estimates were based upon levels of reduction seen for manufacturing steps of several different types of plasma-derived products that were similar in some but not all respects to those used in the manufacture of FVIII products. More high quality data on the levels of vCJD agent clearance achieved during the pdFVIII manufacturing would likely improve the final risk estimate generated by the FDA model. Given the lack of data on vCJD agent clearance for pdFVIII uncertainty is considerable.

Better information on when infectivity is present in human blood during the incubation period is a critical factor in the model, especially if the higher vCJD infection prevalence estimate (of 1 in 4,225) is in the range of the actual vCJD prevalence, and would improve predictions generated by the model. There are no data available on the level of infectious units or $ID_{50}$ units present in the bloodstream of vCJD infected individuals at the time of blood donation. The model extrapolates an estimate of the level of vCJD agent that might be present in human blood based on data from several animal models. However, the presence and level of agent present in an infected individual at the time of blood donation could differ from our assumption and this adds to the uncertainty of the risk assessment outcomes.

The model estimates exposure to the vCJD agent in the form of intravenous $ID_{50}$ units. Data are not available to estimate the probability of various clinical outcomes, such as infection or illness that might be predicted to arise from exposure to a particular level of agent. Although we did estimate a probability of infection in our model, the uncertainty associated with the estimate is considerable. However, a meaningful dose-response model would need to be generated for vCJD exposure in humans to improve estimates of the probability of adverse clinical outcomes for humans. The type of data needed to generate a dose-response model that would improve the quality of TSE risk assessment predictions would necessitate injection of groups of animals at several different concentrations of $ID_{50}$, including low doses below 1 $ID_{50}$ using a protocol that mimics transfusion transmission of vCJD in humans. Both infection and duration of the incubation periods at several different i.v. $ID_{50}$ concentrations would be useful endpoints for developing informative dose-response relationships. Given the state of the current TSE science, estimates of the probability of vCJD infection or illness arising from exposure to the vCJD agent are still extremely uncertain. Nevertheless risk assessment is a tool that provides insight into important factors where additional research is needed into production processes, tools, or strategies that may further reduce vCJD risks and advance product safety for patients.

The manufacturing processes for pdFVIII are highly varied – therefore, any potential clearance of the vCJD agent during production is likely variable and dependent upon the specific steps used to produce the final product. For example, the techniques applied in fractionation process vary from manufacture to manufacture including the sizes of plasma pools used for producing pdFVIII, the yield of products, and the reduction of infectivity during processing varies within a limited range from batch to batch. In addition the utilization of pdFVIII varies from individual to individual. This risk assessment considers the typical production and utilization. Uncertainty from the model should be appreciated. Human plasma-derived FVIII is typically prepared through successive steps of large scale fractionation during the manufacturing process. Cryoprecipitation is the first and a common step in preparation of pdFVIII. Afterward, cryoprecipitate undergoes further fractionation procedures such as precipitation, absorption/desorption, ion exchange and filtration to yield intermediate purity FVIII. In certain cases some hospitals may prepare small amount of cryoprecipitate FVIII from small plasma pools (1-8 donations/pool) for special treatment

purposes. Preliminary risk assessment results indicated that the risk that vCJD would be transmitted through cryoprecipitated AHF is relatively low due to the small size of plasma pool and small numbers of donors involved. This risk assessment uses 3 ranges of possible clearance of vCJD agent from pdFVIII of 2-3 $\log_{10}$, 4-6 $\log_{10}$, and 7-9 $\log_{10}$ to cover the possible ranges for all pdFVIII products presently in the marketplace.

## General comments on model outputs

The risk estimations in this section of the risk assessment are predicated on the assumption that there is homogeneous mixing and dispersion of vials from all pools among all donors. In reality, vials may not be dispensed homogeneously and it is likely that patients draw from only one or a few manufactured lots of pdFVIII product in a given year. FDA did not have data to model this non-homogeneous dispensing of pdFVIII but the model can be used to estimate the average maximal level of i.v. $ID_{50}$ exposure if on a very rare chance all vials used by a patient in a given year happened to contain vCJD agent.

## V. F. Conclusions

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Although donor deferral criteria in place since 1999 have reduced the risk of donation by exposed persons some are not deferred and potentially may donate plasma that contains the vCJD agent. However, the model suggests that the likelihood of a vCJD contaminated plasma pool is low.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the modeling predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from1 in 15,000 for the higher vCJD prevalence estimate and high product usage to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. Due to the wide range of methods used for clearance studies currently available, gaps in information, and the results of the model, it is not possible at this time to determine with any certainty if a specific product may be less or more safe than another.

Although results of the model suggest exposure to vCJD agent is possible, and there is a potential risk of infection that is likely to be very low, it is not possible for the model to provide a *precise* estimate of the vCJD risk in general, or of the actual risk to individual patients. Although the actual risk is highly uncertain, the risk assessment model indicates that the most important factors affecting risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, and the vCJD prevalence in the UK donor population.

*In considering the results of the risk assessment it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products, including pdFVIII.. This includes patients receiving large amounts of other products manufactured from UK plasma donations over a long period of time.* This observation suggests that the actual risk of vCJD infection from pdFVIII is likely to be very low. The absence of cases does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

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# APPENDIX A

## Supplemental technical information for the FDA Risk Assessment

The sections in this appendix provide additional technical information and details of data and modeling approaches used in specific sections (but not all sections) of Section IV. The heading and numbering of each section in this appendix mirrors the sections in "Section IV. Exposure Assessment" portion of the risk assessment.

### A- IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

### A-IV. A. 3. Estimation of age-specific vCJD prevalence based on the age distribution of diagnosed vCJD cases in the UK

Cases of vCJD occur in relatively young individuals (median age of 28 years) compared to classic CJD. Blood and plasma donors are usually at the age 18-40, among whom the vCJD prevalence would be expected to be higher than the prevalence among general population. Because age specific rates of donation and vCJD infection would likely have a large effect on the final risk estimate the FDA model carefully characterizes the age specific prevalence of vCJD and donation rate. Throughout the FDA model, age specific vCJD prevalence rates are calculated for each five year age group beginning at age group of 10 – 14 yrs, 15-19 yrs and so on – and applied in estimating vCJD risk and prevalence for the residents of different geographic regions and the US blood and plasma donors who traveled to those regions. The percentage of reported vCJD cases by age is shown in **Table A-4.3.** The model assumes that the age-specific percentage and prevalence of incubating asymptomatic cases reflects the same age-specific trend as for reported cases of symptomatic vCJD and deaths from vCJD.

**Table A-4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups**

| Age group | <10 | 10-14 | 15-19 | 18-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | >70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Reported vCJD cases in UK (through 2003)[a] (%)** | 0 | 5 (3.4%) | 27 (18.4%) | | 32 (21.8%) | 30 (20.4%) | 22 (14.9%) | 13 (8.8%) | 5 (3.4%) | 3 (2%) | 5 (3.4%) | 0 (0%) | 5 (3.4%) | | |
| **Age distribution of US Source Plasma donors (%)[b]** | 0 | 0 | 0 | 12% | 29.3% | 14.1% | 14.1% | 9.6% | 9.6% | 5.8% | 5.8% | 0% | 0% | 0% | 0% |
| **Age distribution of US Blood (Recovered plasma) donors[c]** | 0 | 0 | 0 | 5% | 13% | 8% | 10% | 12% | 13% | 12% | 11% | 7% | 4% | 5% | 0% |

[a]Hilton *et al.* 2004

[b]Plasma Protein Therapeutics Association (Jan 07, 2005). Where data were organized in broader age group we allocated donor equally among smaller 5 year age groups

[c] Data provided to FDA by Westat in 2002

Some of the general variables for generating age specific estimates from the model are described below.

**Variable:** ***age*** - Age of vCJD cases in 5-year increments

**Variable:** $vCJD_{UK(age)}$ - Reported vCJD cases in the UK by 5-year age groups (through 2003) beginning at 10 – 14 yrs, 15-19 yrs and so on.

**Data used in the model:** Data on the vCJD cases in the UK was derived from Hilton et al. (2004). The data includes cases through the end of 2003.

**Variable:** $Perc_{vCJD(age)}$- Percentage vCJD cases attributed by each age group from 10 – 14 yrs, 15-19 yrs and so on.

**Assumption used in the model:** We assume each of four age groups, 55-59 yrs, 60-64 yrs, 65-69 yrs and 70-74 yrs, contributes same percentage in vCJD cases.

For the four (five-year) age groups from 55-74yrs we assumed an equal percentage of cases were present in each of the four groups since there were very few vCJD cases in this age range. To estimate the percentages for each group we identified five reported cases (specifically, three reported cases in the age-specific prevalence grouping shown in Hilton et al. (2004) for persons aged 55-74 yrs and two cases of blood transfusion vCJD (each > 64 yrs of age) (Llewelyn 2004, Peden 2005)) in the 55-74 yr age range. The five cases in the 55-74 yr old age group are shown in **Table A-4.3**. We assumed an average of 1.25 cases for each of the four age groups from the ages of 55-74 yrs and divided by the total number of vCJD cases for all age groups to get the percentage of cases for each of the four sub age groups, 55-59 yrs, 60-64 yrs, 65-69 yrs and 70-74 yrs.

The percentage of vCJD cases in the UK from each age group is represented by the equation:

$$Perc_{vCJD(age)} = (vCJD_{UK(age)} / \sum_{age=0-4}^{\geq 85} vCJD_{UK(age)}) \times 100\% \qquad \text{(IV. A. 1-1)}$$

## A-IV. A. 3. a. Estimating the UK vCJD prevalence predicted by epidemiological modeling (Clarke and Ghani 2005) for each age group

**Variable:** $vCJD_{since2002}$-Predicted clinical vCJD cases in the UK from 2002 afterward (cases). Predicted clinical cases from 2002 afterward include 32 vCJD cases diagnosed in 2002 and 2003 and 70 (95% CI of 10-190) future cases between 2004 and 2080 predicted by Clarke and Ghani (2005), which give a total of 102 cases (95% CI: 42-222 cases) after 2002.

**Variable:** $Asym\text{-}vCJD_{(age)}$ - Number of asymptomatic vCJD infected individuals from a specific age group in year 2002.

$$Asym\text{-}vCJD_{(age)} = vCJD_{since2002} \times Perc_{vCJD(age)} \qquad \text{(IV.A.2-4)}$$

**Variable:** $Prev_{Asym\text{-}vCJD(age)}$- Prevalence of asymptomatic vCJD infected individuals in the UK by age groups (cases/million).

The prevalence of asymptomatic vCJD cases in the UK by age group is estimated using the equation:

$$Prev_{Asym\text{-}vCJD(age)} = Asym\text{-}_{vCJD(age)}/Pop_{UK(age)} \quad \text{(IV.A.2-5)}$$

## A-IV. A. 3. b. Estimating the UK vCJD prevalence derived from tissue surveillance for each age group

For the risk assessment model we converted the 3 in 12,674 presumptive positive rate to an average rate of vCJD in the UK population of 1 in 4,225 (and used the 1 / 20,300 to 1 / 1,450 at 95% CI; proportions were converted from the 95% CI reported by Hilton et al (2004)). Demographic information of reported vCJD cases (**Table A-4.3**) indicated that the younger population (20 -29 yrs of age) that was deliberately oversampled in this study may have been more susceptible to the disease. The vCJD prevalence among UK donors might, therefore, be over-represented by the prevalence of 20-29 years age group derived from the surveillance study. Assuming the sensitivity and specificity of the testing method is 100%, the estimated rate of 1 in 4,225 translates roughly to a vCJD prevalence of 237 cases per million (95% CI: 49 – 692 cases per million) for all age groups. The authors (Hilton et al 2005) indicated that approximately 60% of the samples tested (from 7,600 patients) came from patients 20-29 years of age. Among the 20-29 year old group we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million.

We then derived the prevalences for the remainder of the UK donor population by determining the proportional difference between the vCJD prevalence from the tissue study group and the number of actual reported vCJD cases for donors in the 20-29 years age group. This proportion was then applied to the remaining age groups in the distribution of reported vCJD cases to determine the prevalence for each age group. By multiplying our extrapolated vCJD prevalence for incubating cases by the total donor population we were able to estimate the number of possible incubating vCJD cases in each US donor age group. We assumed that a plasma pool used to manufacture pdFVIII product in the US in the year 2002 consisted 6,000 to 360,000 donations, and several donations in the pool likely came from the same donor. The estimated prevalence was then used to generate variables and parameters representing the potential number of vCJD donors or donations that might be present in a plasma pool.

**Variable:** $Prev_{Asym\text{-}vCJD(20\text{-}30)}$ Prevalence of asymptomatic vCJD infected individuals in the UK 20-30 year old age group (cases/million)

**Assumptions used in the model:** The vCJD infectious agent is present in the blood of the individual when the the accumulation of prion protein can be detected in lymphoreticular tissue. Prevalence of vCJD asymptomatic individuals in the UK 20-30 year old age group is likely to be 400 cases/million, 95% CI=100-1200 cases/million. The values for this variable were estimated from the Hilton *et al* studies (2000, 2002, 2004).

**Variable:** $Pop_{UK(age)}$- Population in the UK by age groups (Thousands).

**Data used in the model:** The data for UK population were sourced from UK government statistics (UK National Statistics, 2005). Where UK data were organized in broader categories of 10 to 15 years we allocated population equally among smaller 5 year age groups.

**Variable:** $Asym\text{-}vCJD_{(20\text{-}30)}$ - The number of asymptomatic vCJD infected individuals in the 20-30 yr-old UK age group. This variable is represented by the equation:

$$Asym\text{-}vCJD_{(20\text{-}30)} = Prev_{vCJD(20\text{-}30)} \times Pop_{UK(20\text{-}30)} \quad \text{(IV.A.2-1)}$$

**Variable:** $Asym\text{-}vCJD_{(age)}$ - Number of asymptomatic vCJD infected individuals in the UK by age groups

**Assumptions used in the model:** Number of asymptomatic vCJD infected individuals from an age group is proportional to the percentage of reported vCJD cases from that age group. The age distribution of asymptomatic vCJD cases was assumed to be the same as that of symptomatic cases.

The number of asymptomatic vCJD individuals in the UK per age group was estimated using the following equation:

$$Asym\text{-}vCJD_{(age)} = Asym\text{-}vCJD_{(20\text{-}30)} \times (Perc_{vCJD(age)} / Perc_{vCJD(20\text{-}30)}) \quad \text{(IV.A.2-2)}$$

**Variable:** $Prev_{Asym\text{-}vCJD(age)}$- Prevalence of asymptomatic vCJD infected individuals in the UK by age groups (cases/million).

The prevalence of asymptomatic vCJD cases in the UK by age group is estimated by the equation:

$$Prev_{Asym\text{-}vCJD(age)} = Asym\text{-}_{vCJD(age)} / Pop_{UK(age)} \quad \text{(IV.A.2-3)}$$

## A-IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2)

### A-IV. B. 1. a. Annual US plasma donors and characterization by age

### A-IV. B. 1. b. Source Plasma collection in the United States: characterized by donor age

**Variable:** $DN_S$ – Annual number of Source Plasma units used to make pdFVIII .

**Assumption used in the model:** It was assumed that, on average, 3.3 million units of Source Plasma were used in each year to make pdVIII. It was further assumed that there is a 10% standard deviation in the number of Source Plasma units used to make pdFVIII for any given year.

**Data used in the model:** The annual number of Source Plasma units was back calculated based on annual units of pdVIII product made from Source Plasma, the average yield of pdFVIII (187 units per liter

plasma) and average volume of single unit of Source Plasma (700 ml per unit). The information on annual units of pdFVIII made from plasma collected in the US, yield of factor VIII and unit volume of plasma were collected from pdFVIII manufacturers.

**Variable:** $DR_S$–Annual number of donors who contribute Source Plasma for manufacture of pdFVIII.

**Assumption used in the model:** It was assumed that there are approximately 1 million Source Plasma donors in the US each year. It was further assumed that Source Plasma from any individual donor may be used to make pdFVIII. Therefore, we calculated that there were approximately 1 million donors who contributed Source Plasma for the manufacture of pdFVIII. It was further assumed that there could be a 10% standard deviation in the number of donors in any given year.

**Variable:** *Age* – Age information for US plasma donors was grouped in a two year increment for 18-19 years old because the model assumed that 18 was the minimum age of donation. The remaining population was grouped by 5-year increments – including 20- 24yrs old, 25-29yrs old, and so on

**Variable:** $DR_{S\text{-}perc(age)}$ - The percentage of Source Plasma donors from a given age group.

**Data used in the model:** Distribution of US Source Plasma donors by age was obtained from the Plasma Protein Therapeutics Association (2005). Where data (PPTA, 2005) were organized in broader age groups of 10 years or 15 years, we generated 5-year age subgroups by allocating the percentage equally among each subgroup.

**Variable:** $DR_{S(age)}$ – The annual Source Plasma donors by age groups who contribute plasma for pdFVIII manufacturing is represented by the equation:

$$DR_{S(age)} = DR_S \times DR_{S-perc(age)} \qquad \text{(IV.B.1-1)}$$

**A-IV. B. 1. c. Recovered plasma collection in the United States: Characterized by donor age**

**Variable:** $DN_R$ - Annual units of recovered plasma used to make pdFVIII.

**Assumption used in the model:** It was assumed that approximately 1,800,000 units of recovered plasma are used to make pdFVIII annually. This estimation was generated by backcalculation beginning with the total quantity of pdFVIII manufactured in the US. It was further assumed that there was a 10% standard deviation in the number of units for any given year.

**Data used in the model:** The annual number of total units of pdFVIII manufactured from recovered plasma collected in the US was estimated by back calculation. The calculation was based on the total quantity of annual units of pdVIII product made from recovered plasma collected in the US. We can further estimate the number of donations used to make the pdFVIII from recovered plasma using estimates in the literature for the average yield of pdFVIII 187 units per liter of plasma (WFH, 2004) and average volume of single unit of recovered plasma (200 ml per unit). The information on annual units of pdFVIII made from plasma collected in the US was collected from pdVIII manufacturers.

**Variable:** $DN_{Bl\text{-}perc(age)}$ – The percentage of blood units donated by a given age group.

**Data used in the model:** Distribution of blood units by donor age group was obtained from Westat data provided to FDA in 2002 (Data shown in A-4.3).

**Variable:** $DN_{R(age)}$ – Annual units of recovered plasma used to make pdFVIII by donor age group

$$DN_{R(age)} = DN_R \times DN_{Bl-perc(age)} \quad \text{(IV.B.2-1)}$$

**Variable:** $DR_{R(age)}$ – Annual number of donors by age group who contribute recovered plasma that is used for manufacture of pdFVIII

**Assumption used in the model:** Each unit of recovered plasma used to make pdFVIII comes from different donors. Therefore, number of donors from an age group equals the number of donations from that age group.

The annual number of recovered plasma donors by age group was calculated using the equation:

$$DR_{R(age)} = DN_{R(age)} \quad \text{(IV.B.2-2)}$$

**Variable:** $DR_R$ - The annual total of potential recovered plasma donors who contribute the plasma that is used for manufacture of pdFVIII, which was estimated in the model using the summation function:

$$DR_R = \sum_{age=18-19}^{70-74} DR_{R(age)} \quad \text{(IV.B.2-3)}$$

## A-IV. B. 2. Total plasma donors and donations- for manufacture of pdFVIII in the US

**Variable:** $DR_{Tot}$ - The annual total of potential plasma donors who contribute plasma for pdFVIII manufacturing is estimated by summing the number of Source Plasma donors and recovered plasma donors and is represented by the equation:

$$DR_{Tot} = DR_S + DR_R \quad \text{(IV.B.3-1)}$$

**Variable:** $DN_{Tot}$ - The annual total of potential plasma units used to make pdFVIII is estimated by summing the number of Source Plasma donations and recovered plasma donations and is represented by the equation:

$$DN_{Tot} = DN_S + DN_R \quad \text{(IV.B.3-2)}$$

## A-IV. C. Estimation of the probability that a plasma pool may contain a donation from an infected donor that contains vCJD agent

## IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood

## A-IV. C. 1. a. US plasma donors with history of travel to the UK: Number of donors potentially infected and vCJD agent is present in the blood

## A-IV. C. 1. a. i. US plasma donors with history of travel to the UK: Percentage of donors and duration of travel

The risk of vCJD infection in US plasma donors is a function of the intensity of exposure to the BSE agent. The intensity of exposure is assumed to be proportional to the amount of time spent, or duration of travel, in the UK and the prevalence of BSE in UK cattle during the period from 1980 – 1996. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 3 months) in the UK during 1980-1996, and to derive the frequencies for various durations of travel for 3 months or more. The period of 3 months or more corresponds to the length of time in the current policy that defers donors that traveled to or resided in the UK. The travel survey data on blood donors pose a limitation because the survey was conducted on whole blood donors and may not exactly reflect the travel histories of plasma donors. Unfortunately, to our knowledge there is not travel data available on plasma donors. Therefore, we assumed that plasma donor travel characteristics to the UK and other countries in Europe since 1980 are similar to those of whole blood donors and used this information in the FDA risk assessment. Some may argue that plasma donors travel less frequently than their blood donor counterparts so use of data on blood donors may overestimate the risk.

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC 2000).

**Variable:** $i$ - The duration interval used to group donors who traveled to the UK from 1980-1996 based on the quantity of time spent in the UK during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (in months) for interval $i$ representing the duration of travel or residence by US donors in the UK during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}UKi}$ – The cumulative percentage of blood donors who traveled to the UK within duration interval $i$ or longer.

**Variable:** $Perc_{BIDR\text{-}UKi}$ - Percentage of blood donors who traveled to the UK within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}UKi}$

**Variable:** $Perc_{BIDR\text{-}UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval *i* among all donors who have ever traveled to the UK is represented by the equation:

$$Perc_{BIDR-UKi/UK} = (Perc_{BIDR-UKi} / CumPerc_{BIDR-UK,i>1day-1month}) \times 100\% \qquad \text{(IV.C.1.a-1)}$$

## A-IV.C.1.a.ii. All US plasma donors with a history of travel to the UK: Percentage and number of donors in each age group by year and duration of travel

For the purposes of our analyses we grouped all donors and donors who traveled to the UK between 1980 and 1996 into age groups of five year increments (20 – 24yrs, 25 – 29 yrs, etc). Because the minimum age of donation is 18, the model also included the donor group 18 & 19 years of age. The percentage of donors in each age group who traveled to the UK between 1980 and 1996 was calculated based on the total annual number donors who traveled to the UK between 1980 and 1996 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel.

As mentioned earlier, data are lacking on the travel characteristics of plasma donors, so the FDA used travel survey data collected from blood donors to estimate past travel history. Because plasma donors are less likely to travel use of these data may yield an overestimate of the actual risk. Characteristics of blood donors on travel including the percentage of donors from each age group who traveled to the UK during period between 1980 and 1996, and distribution of donor travel by duration were applied to plasma donors for estimation of the number of plasma donors from each age group who have traveled or resided in the UK from 1980 to 1996 for specific periods of time ranging from less than 3 months to greater than 5 years duration. Furthermore, the model used data that detailed the number of annual visits of US travelers to the UK to allocate donor travel specifically to an individual calendar year.

- **Calculation of the annual number of blood donors who traveled to the UK from 1980 through 1996**

Blood donors donate whole blood and soon after the liquid plasma portion of whole blood is separated and plasma is called recovered plasma. The model assumes that approximately 200 mls of recovered plasma are produced from a unit of whole blood.

**Variable :** $DR_{BI}$-The annual total number of potential blood donors in the US per year

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR\text{-}UK}$ - Percentage of US blood donors who traveled to the UK during the period from 1980 through 1996.

**Data used in the model:** Approximately, 22.5% of US blood donors have a history of travel to the UK any time during the period from 1980 through 1996, according to data contained in the National Blood Donor Travel Survey (TSEAC 2000).

**Variable:** $DR_{BI\text{-}UK}$ -Total number of blood donors estimated to have traveled to the UK from 1980 through 1996

$$DR_{BI-UK} = DR_{BI} \times Perc_{BIDR-UK} \quad \text{(IV.C.1.a-2)}$$

- **Calculation of the percentage of blood donors for each specific age group**

**Variable:** *Age*- Age of donors grouped in 5 year increments (e.g., 20 – 24 yrs, 25 – 29yrs, etc.) per year and 18-19 yr old age cohort.

**Variable:** $Perc_{BIDR(age)}$-Percentage blood donors in a specific age group.

**Variable:** ***BLDR(age)***- Annual number of blood donors in a specific age group

$$Perc_{BLDR(age)} = BLDR(age) / \sum_{age=18-19}^{65-69} BLDR(age) \quad \text{(IV.C.1.a-3)}$$

**Variable:** ***BLDN***-Annual total number of blood donations in the US.

**Variable:** $Perc_{BIDN(age)}$- Percentage of blood donations in each specific age group of US donors.

**Data used in the model:** Percentage of blood donations by age group was obtained from Westat (2002).

**Variable:** $Freq_{BIDN(age)}$-Average annual number of blood donations from a donor in a specific age group

**Data used in the model:** Information on the average annual number of donations bydonors in each specific age group was obtained from Westat (2002).

$$BLDR(age) = BLDN \times Perc_{BLDN(age)} / Freq_{DN(age)} \quad \text{(IV.C.1.a-4)}$$

Replace *BLDR(age)* in equation IV.C.1.a-3 with equation IV.C.1.a.-4, resulting in the expression:

$$Perc_{BIDR(age)} = (Perc_{BIDN(age)} / Freq_{BIDN(age)}) / \sum_{age=18-19}^{65-69} (Perc_{BIDN(age)} / Freq_{BIDN(age)}) \quad \text{(IV.C.1.a-5)}$$

**Variable :** $DR_{BI}$-The annual total number of blood donors in the US, which is assumed to be 8 million.

**Variable:** $DR_{BI(age)}$- The annual number of blood donors in each five-year age group and the18-19 yr old age cohort.

$$DR_{BI(age)} = DR_{BI} \times Perc_{BIDR(age)} \quad \text{(IV.C.1.a-6)}$$

**Variable:** $DR_{BI\text{-}UK}$ -Total number of US blood donors who have traveled to the UK during the period 1980 - 1996

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ - The percentage of US blood donors in an age group who traveled to the UK during the period 1980 - 1996.

$$DR_{BI-UK} = \sum_{age=18-19}^{65-69}(Perc_{BIDR-UK(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.a-7)}$$

**Variable:** $Odd_{T(age)}$- Age specific odd ratios for travel compared to the age group 18-19 years.

**Data used in the model:** The odds ratios for likelihood of travel for each age group were derived from the travel data obtained from 1980-1996 blood donor travel survey. An odds ratio of 1 was assigned to the donor group aged 18-19 years. The odds ratios for other age groups is a function of the travel frequency of those age groups compared to the travel frequency of the age group of 18-19 years

$$Perc_{BIDR-UK(age)} = Odd_{T(age)} \times Perc_{BIDR-UK(18-19)} \qquad \text{(IV.C.1.a-8)}$$

Replacing the variable, $Perc_{BIDR\text{-}UK(age)}$, in equation IV.C.1.a-7 with equation IV.C.1.a-8 the percentage of blood donors in the age group of 18-19 years of age who traveled to the UK was calculated using the following equation:

$$Perc_{BIDR-UK(18-19)} = DR_{BI-UK} / \sum_{age=18-19}^{65-69}(Odd_{T(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.a-9)}$$

## A-IV. C. 1. a. ii. a. Number of US Source Plasma donors who traveled to the UK in a specific year from 1980 to 1996 and by age group

**Variable:** *age* - Age of US plasma donors in groups by 5-year increments and 18-19 yr old age cohort.

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B. 1.) - The annual number of donors by age group who contribute Source Plasma to make pdFVIII.

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ (calculated in section A-IV.C.1.a.i.b.) - the percentage of blood donors in each age group who traveled to the UK between 1980-1996.

**Variable:** $DR_{S\text{-}UK(age)}$ - Number of Source Plasma donors who traveled to the UK from 1980 through 1996 by age groups.

**Assumptions used in the model:** The percentage of Source Plasma donors who traveled to the UK is the same as the percentage of blood donors of the same age group.

The number of Source Plasma donors who traveled to the UK from 1980 to 1996 by age group is represented by:

$$DR_{S-UK(age)} = DR_{S(age)} \times Perc_{BIDR-UK} \quad \text{(IV.C.1.a-8)}$$

The risk of vCJD infection in US donors who traveled to the UK was assumed proportional to the relative level of exposure to the BSE agent in each year through the UK food supply. The proportional risk was derived based on the number of BSE-reported cattle in a given year from 1980 through 1996 divided by the total number of BSE cases to date. The model generates categories (or bins) for Source Plasma donors by year of travel and estimates the risk more accurately by incorporating the information about proportional risk due to the BSE epidemic in the UK

In 1996 after the emergence of human vCJD cases the UK government implemented stringent food chain controls that decreased the number of high risk animals and high risk tissues containing BSE agent from entering the human food supply. In the early 1980s human exposure may have begun at a low level as BSE spread among the UK cattle population. The BSE epidemic expanded throughout the 1980s and peaked in 1992, then risk started to decrease as animal feed measures were implemented and more stringent human food chain controls were implemented in 1996. Today, a few hundred cases of BSE are identified in the UK annually – but it is unlikely that the animals or products from BSE-infected animals in the UK enter the human food supply. The model incorporates the changing dynamics of the BSE epidemic since 1980 and accounts for relative changes in the levels of human, specifically US plasma donors, exposure to the BSE agent and possible vCJD infection.

**Variable:** $y$ - Calendar year of travel.

**Variable:** $V_y$ - Number of visits by year to the UK by US travelers (in thousands)

**Data used in the model:** Number of visits by year to the UK by US travelers (UK Government Statistics, 2005).

The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

**Assumption used in the model**: US Source Plasma donors have similar travel patterns to the US blood donor population, which is assumed to be similar for the larger US population.

**Assumption used in the model**: It was assumed that no US traveler visited the UK more than once per year. This may potentially overestimate the vCJD risk for US plasma donors (because repeat travel by the

same donor is not addressed) and underestimate it in certain other cases (travelers who visit multiple times per year). FDA found no data that quantified the numbers of multiple visits or repeat visits by the same traveler that likely occurred for US donors with a history of UK travel.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 is represented by the equation:

$$V_{y/1996} = V_y / V_{1996} \quad \text{(IV.C.1.a-9)}$$

**Variable:** ***age*** - Age of US plasma donors in groups of 5-year increments (e.g., 20-24 yrs, etc.) and 18-19 yr old age cohort..

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B. 1.) - the annual Source Plasma donations by age groups.

**Variable:** $DR_{S\text{-}UK(age)}$ - Number of Source Plasma donors who traveled to the UK from 1980 through 1996 by age group in five-year increments and 18-19 yr old age cohort.

**Assumptions used in the model:**

- The same percentage of Source Plasma donors traveled to UK as blood donors
- Frequencies of travels are similar among the donors of different age groups.
- Travel rates for the general US population are the same as the travel rate for blood and plasma donors.

The number of Source Plasma donors who traveled to the UK from 1980 to 1996 by age group is represented by:

$$DR_{S\text{-}UK(age)} = DR_{S(age)} \times Perc_{DR\text{-}UK} \quad \text{(IV.C.1.a-5)}$$

**Variable:** $DR_{S\text{-}UK(age),y}$ - the number of Source Plasma donors who traveled to the UK in year $y$ by age group

Source Plasma donors with a history of travel to the UK among each age group ($DR_{S\text{-}UK(age)}$) was allocated to individual travel years based on the yearly distribution of visits to the UK by US travelers (UK National Statistics, 2005). The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

**Assumption used in the model**: US Source Plasma donors have similar travel patterns as the general US population and US blood donors.

The number of US Source Plasma donors who have traveled to the UK in year y between 1980-1996 is represented by the equation:

$$DR_{S-UK(age)y} = DR_{S-UK(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.a-10)}$$

## A-IV. C. 1. a. ii. b. US Source Plasma donors with history of travel to the UK: Duration of travel by age group

There were no data that we are aware of that details the travel histories of Source Plasma donors in the US. Travel data for US blood donors was used to estimate travel patterns for Source Plasma donors after an adjustment for the frequency of travel based on the age of Source Plasma donors and the age-specific odds ratios for travel, which was obtained from 1980-1996 Blood Donor Travel Survey (TSEAC, 2000). The model used the data on the number of Source Plasma donors who have traveled to the UK in a specific year and subdivided those individuals into additional categories based on estimated duration of stay. The categories of duration of stay was estimated based on the Blood Donor Travel Survey (TSEAC, 2000). Use of blood donor travel data in the model implicitly assumes that travel rates are similar for both blood and plasma donors. However, it is thought that plasma donors likely travel less and are therefore at lower risk for vCJD, therefore the model results may overestimate the potential vCJD risk for plasma donors.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to UK from 1980-1996 based on the time they spent in the UK (same variable used above in section A-IV. C. 1. a. i.).

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1. i.).

**Variable:** $DR_{S\text{-}UK(age),y}$ - the number of Source Plasma donors who traveled to the UK in year $y$ by age group (calculated in A-IV. C. 1. a. ii. (a))

**Variable:** $Perc_{BIDR\text{-}UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to the UK (calculated in A-IV. C. 1. a. i. a)

**Variable:** $DR_{S\text{-}UK(age)y,i}$ - Number of Source Plasma donors within a specific age group that traveled to the UK in year $y$ for a duration of $i$ and is represented by the equation:

$$DR_{S-UK(age)y,i} = DR_{S-UK(age)y} \times Perc_{DR-UKi/UK} \qquad \text{(IV.C.1.a-11)}$$

## A-IV. C. 1. a. ii. c. Number of US recovered plasma donors with a history of travel to the UK in a specific year from 1980 – 1996 by age group

Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year (or once every 54 days)– and on average we assumed a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable:** $y$ **– year of travel** (same variable used above in section A-IV. C. 1. a. ii. a) to the UK during the period 1980 - 1996 by US plasma donors.

**Variable: age – age groups of the population in five-year increments** (same variable used above in section A-IV. B. 1.).

**Variable:** $DR_{R(age)}$ - number of potential recovered plasma donors per year by age group (described in section A-IV. B. 2).

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ (calculated in section A-IV.C.1.a.i.b.) - the percentage blood donors in each specific age group who have traveled to the UK between 1980-1996.

**Variable:** $DR_{R\text{-}UK(age)}$ – The number of recovered plasma donors who have traveled to the UK from 1980 through 1996 by age group and is represented by the equation:

$$DR_{R-UK(age)} = DR_{R(age)} \times Perc_{BIDR-UK(age)} \qquad \text{(IV.C.1.a-12)}$$

The model categorizes US recovered plasma donors by year of travel in order to estimate the potential vCJD risk more accurately by incorporating the information about BSE epidemic in the UK. The number of beef cattle affected each year by the BSE epidemic in the UK changed throughout the epidemic. Donors who visited the UK at the height of the BSE epidemic in 1992, we assumed, were more likely to be exposed to BSE agent (and vCJD risk) than a donor who visited in 1996 after food chain controls were implemented. The model accounts for this changing exposure potential by analyzing US donors who may have traveled to the UK for each year from 1980 through 1996. The number of recovered plasma donors who traveled to the UK was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers determined from UK travel data.

**Variable:** $DR_{R\text{-}UK(age)}$ - Number of recovered plasma donors traveled to the UK from 1980 through 1996 by age groups (calculated above).

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a. ii. a.).

**Data used in the model:** Number of visits by year to the UK by US travelers was determined using UK Government Statistics (2005).

**Assumption about variable:** US recovered plasma donors have a similar travel pattern as the US total population. The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during

the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

The number of recovered plasma donors who traveled to the UK in year *y* by age groups during the period from 1980-1996 is represented by the equation:

$$DR_{R\text{-}UK(age)y} = DR_{R\text{-}UK(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.a-13)}$$

## A-IV. C. 1. a. ii. d. US recovered plasma donors with history of travel to the UK: Duration of travel by age group

Recovered plasma donors who traveled to the UK in a specific year ($DR_{R\text{-}UK(age)y}$) in the years 1980-1996 were further partitioned in the model into subgroups or "bins" based on travel duration and by 5-year age groups and 18-19 yr old age cohort. Data on the percentage of blood donors who traveled to the UK since 1980 for a certain duration(s) (TSEAC 2000) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to the UK from 1980-1996 based on the time they spent in the UK (same variable used above in section A-IV. C. 1.)

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R\text{-}UK(age)y}$ - The number of recovered plasma donors who traveled to the UK in year *y* by age group (calculated in A-IV. C. 1. a. ii. c)

**Variable:** $Perc_{BIDR\text{-}UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to the UK (calculated in A-IV. C. 1. a. i. a)

**Variable:** $DR_{R\text{-}UK(age)y,i}$ - The number of recovered plasma donors among an age group who have traveled to the UK in year, *y* ,for duration of $i$ and represented by the expression:

$$DR_{R\text{-}UK(age)y,i} = DR_{R\text{-}UK(age)y} \times Perc_{DR\text{-}UKi/UK} \qquad \text{(IV.C.1.a-14)}$$

## A-IV. C. 1. a. iii. US plasma donors with a history of travel to the UK: Adjustment of relative risk to account for variations in BSE risk by specific year and travel duration

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because the BSE epidemic in UK cattle and exposure of the human population to the BSE agent in the UK was greater than any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence, which is difficult to estimate. The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (TSEAC 2004). BSE was first diagnosed in the United Kingdom in 1986 and the epidemic peaked in 1992, a year when the risk of exposure to the BSE agent would have likely been highest for residents and visitors to the UK. Human exposure risk to the BSE agent would likely have decreased dramatically in 1996 with the culling of animals over 30 months of age from the food production system and the institution of food chain controls to prevent high risk tissues that might contain BSE agent from entering the food and animal food supplies. Presumably there were dramatic variations in the BSE exposure risk, and hence, the human vCJD infection risk that occurred from year to year. Therefore, the model adjusted the vCJD risk for US plasma donors with a history of extended travel or residence in the UK by multiplying by the proportional BSE risk per year (e.g., the BSE exposure risk in a given year compared to the total BSE risk since 1980). Additionally, the model included calculations on the estimated duration of UK travel or residence by US plasma donors based on US donor survey data (TSEAC 2000) to generate a more accurate vCJD risk estimate.

### A-IV. C. 1. a. iii. a. Variant CJD risk for individual UK residents from 1980 through 1996

**Variable:** $R_{UK}$- The accumulated vCJD risk per UK resident from 1980 through 1996.

**Assumption used in the model:** The UK population has the highest risk of exposure to BSE or vCJD, we assumed the average accumulated risk per UK individual is 1. Also, the relative risk for UK residents is 1, which is equivalent to the UK vCJD prevalence.

### A-IV. C. 1. a. iii. b. US plasma donors with a history of travel to the UK: Adjustment for the proportional individual BSE exposure risk for the UK population per year between 1980 to 1996.

The model calculates the risk and potential magnitude of BSE exposure for donors, in any given year in the UK since 1980, as a function of the number of BSE cases in a specific year divided by the total of all BSE cases since 1980.

**Variable:** $y$ – year of travel (same as variable used above in section A-IV. C. 1. a. ii. A.) by US plasma donor to the UK from 1980 to 1996.

**Variable:** $BSE_{UKy}$- The annual number of reported BSE cases in the UK since 1986 (OIE, 2005).

**Variable:** $R_{UKy}$- Proportional BSE exposure risk in the UK by specific year from 1980 to 1996.

**Assumptions used in the model:**

- The BSE exposure risk, and hence, most of the vCJD risk in the UK occurred largely between 1980 and 1996.
- The vCJD risk in the UK was assumed to be negligible after 1996, when stringent food chain controls were put in place to prevent contamination of beef with high risk tissue.
- The yearly rate of the human exposure risk to the BSE agent in the UK is proportional to the number of reported BSE annual cases in the UK
- The vCJD risk is additive for each year of residency during the specific time period.
- A person residing for five or more years during the time period between 1980 and 1996 in the UK is assumed to have a relative risk of 1 (or 100%), i.e., a probability of vCJD infection that is the same as that of the entire UK population.

The proportional BSE risk in the UK per specific year prior to 1997 is represented by the equation:

$$R_{UKy} = R_{UK} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.a-15)}$$

## A-IV. C. 1. a. iii. c. US plasma donors with a history of travel to the UK: BSE exposure risk and vCJD risk in year *y* for a period of *i*, during the period from 1980 to 1996.

**Variable:** $R_{DR\text{-}UKy,i}$- The potential vCJD risk of an individual US donor who traveled to the UK in specific year during the period 1980-1996 for a specific duration.

The potential vCJD risk for the US plasma donor subpopulation that traveled to the UK in a specific year for a specific duration was calculated using a pro-rated monthly rate, which was calculated based on the proportional BSE exposure risk in the UK in the specific year. The blood donor travel survey conducted by the American Red Cross (TSEAC 2000) collected data on the accumulated stay of donors in the UK from 1980 through 1996, which, for simplicity, was assumed to be the duration of a single, consecutive stay, when calculating the risk.

**Assumptions used in the model:**

- Risk of vCJD infection is proportional to the duration of the stay in the UK during the period 1980-1996
- All travelers evaluated completed a single, consecutive stay

As mentioned earlier, any US plasma donor with 5 years or more of accumulated stay in the UK is assumed to have average risk of 1, a risk equal to the average risk of an UK resident and equal to the UK vCJD prevalence.

The BSE exposure risk for US plasma donors with a stay less than or equal to one year – is represented by the equation:

$$R_{DR\text{-}UKy,i} = (R_{UKy} / 12) \times D_i \qquad \text{(IV.C.1.a-16)}$$

for $i_{upper} \leq 1$ years;

The BSE exposure risk for US plasma donors with a stay less than five years but greater than or equal to one year is represented by the equation:

$$R_{DR\text{-}UKy,i} = (Average(R_{UKy} : R_{UK(y+Roundup(i_{upper},0))}) / 12) \times D_i \quad \text{(IV.C.1.a-17)}$$

for 5 years $< i_{upper} \geq 1$ year;

The BSE exposure risk for US plasma donors with a stay greater than or equal to five – is represented by the equation:

$$R_{DR\text{-}UKy,i} = 1 \quad \text{(IV.C.1.a-18)}$$

for $i_{upper} \geq 5$ years

## A-IV. C. 1. a. iv. US plasma donors with a history of travel to the UK: Probability of potential infection with vCJD based on duration of travel to the UK and age

This section describes the portion of the model that estimates the probability that a US plasma donor in a specific age group, who traveled to the UK for a specific duration during the time-span of 1980 through 1996, was infected with vCJD.

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ – the probability of vCJD infection per individual UK resident of a specific age group

**Variable:** $Prev_{Asym\text{-}vCJD(age)}$- Prevalence of asymptomatic vCJD infection in the UK for each age groups in five-year increments (e.g., 20-24 yrs, etc.) and the 18-19yr old group (calculated in A-IV.A.3.b.).

$$Pr_{vCJD\text{-}UK(age)} = Prev_{Asym\text{-}vCJD(age)} / 1000000 \quad \text{(IV.C.1.a-19)}$$

**Variable:** $Pr_{vCJD\text{-}DR\text{-}UK(age)y,i}$ – The probability of infection for individual US plasma donor of a specific age group who had traveled to the UK in a specific year for a specific duration

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$Pr_{vCJD-DR-UK(age)y,i} = Pr_{vCJD-UK(age)} \times R_{DR-UKy,i} \quad \text{(IV.C.1.a-20)}$$

## A-IV. C. 1. a. v. Number of all US pdFVIII plasma donors with history of travel to the UK and potentially infected with vCJD

This section of the model estimates the total number of all US plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996. To derive the total number of donors the model separately estimates the number of potentially infected Source Plasma donors and potentially infected recovered plasma donors (described in the subsequent sections below) and sums the two.

**A-IV. C. 1. a. v. a. Number US Source Plasma donors with history of travel to the UK and potentially infected with vCJD during travel to the UK**

Plasma is collected from Source Plasma donors in a process called plasmapheresis in which an average of approximately 700 milliliters of plasma are collected. Source Plasma donors donate an average of 14 times per year, but can donate up to 48 times per year.

This component of the model estimates the number of US Source Plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}UK(age)y,i}$- Number of Source Plasma donors potentially infected with vCJD during travel to the UK during 1980-1996 by age, year and duration of travel.

$$DR_{vCJD-S-UK(age)y,i} = Bionomial(DR_{S-UK(age)y,i}, \Pr_{vCJD-DR-UK(age)y,i}) \quad \text{(IV.C.1.a-21)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UKy}$ - Number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK.

$$DR_{vCJD-S-UK} = \sum_{Age=18-19yrs\,i=1day-3months}^{50-54yrs} \sum^{>=5years} DR_{vCJD-S-UK(age)y,i} \quad \text{(IV.C.1.a-22)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Defy}$- Number of Source Plasma donors potentially infected with vCJD in year y and not deferred by current policy.

$$DR_{vCJD-S-UK-defy} = \sum_{Age=18-19yrs\,i=3-5months}^{50-45yrs} \sum^{>=5years} DR_{vCJD-S-UK(age)y,i} \quad \text{(IV.C.1.a-23)}$$

Current deferral policy (FDA 2002) defers individuals who have history of travel to the UK from 1980 through 1996 for an accumulated residence of 3 months or more from donating blood and plasma. The number of potentially infected donors who meet deferral criteria was calculated by equation:

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Res}$- Residual risk due to the number of Source Plasma donors potentially infected with vCJD not deferred by current policy

$$DR_{vCJD-S-UK-Resy} = \sum_{Age=18-19}^{50-54yrs} DR_{vCJD-S-UK(age)y,i=1day-3months} \quad \text{(IV.C.1.a-24)}$$

## A-IV. C. 1. a. v. b. Number of US Source Plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

Perhaps the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD-infected donor that contained infectious vCJD agent in their blood at the time of

donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of Source Plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $y$ - The calendar year in which a plasma donor traveled and infected with vCJD.

**Assumption used in the model:** This risk assessment assesses the risk for pdFVIII product made in 2002.

**Variable:** $T_{Inf\text{-}2002y}$ - Time Period between infection/travel and year of 2002 when the plasma was collected

**Variable:** $Pr\text{-}LH_y$ - Probability the disease is in the last half incubation period of the disease (and donor is prionemic), if infected in year y

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected.

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.a-25)}$$

For an individual to have vCJD agent present in their blood and plasma (prionemic) in 2002, the elapsed period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or greater than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent the time between events, in this case the time from infection to the occurrence of symptomatic disease. The distribution is defined by two parameters, one that produces the shape of the curve and a second that generates the scale between events, which in this case is the mean incubation period of 14 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual has vCJD agent present in their blood and plasma in year 2002 (the baseline year of the model) was calculated by the expression:

Cumulative frequency of Gamma (4.7, 3.6), at x = 2×(1997-y)

**Variable:** $DR_{vCJD\text{-}S\text{-}UKy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK.

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and were in the last half incubation period of the disease in 2002 at the time of donation.

$$DR_{vCJD-S-UK-LHy} = Binomial(DR_{vCJD-S-UKy,}\ \mathrm{Pr}_{LH-y}) \qquad \text{(IV.C.1.a-26)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}defy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and met deferral criteria

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}def\text{-}LHy}$ - Total number of Source Plasma donors in the last half incubation period of the disease (prionemic) and met current deferral criteria (FDA 2002).

$$DR_{vCJD-S-UK-Def-LHy} = Binomial(DR_{vCJD-S-UK-Defy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.a-27)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Resy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and did not meet deferral criteria

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}LHy}$ - Total number of Source Plasma donors in the last half of the incubation period of the disease who did not meet current deferral criteria (FDA 2002).

$$DR_{vCJD-S-UK-Res-LHy} = Binomial(DR_{vCJD-S-UK-Resy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.a-28)}$$

## A-IV. C. 1. a. v. c. Number of US recovered plasma donors with history of travel to the UK and potentially infected with vCJD

Recovered plasma donors donate whole blood from which the plasma is separated out (or recovered). Like blood donors recovered plasma donors donate an average of 1.7 times per year but can donate up to 6 times per year. The model assumes the average amount of plasma in a recovered plasma unit is approximately 200 milliliters.

This component of the model estimates the number of US recovered plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}R\text{-}UK(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to the UK during 1980-1996 by age, year and duration of travel

$$DR_{vCJD-R-UK(age)y,i} = Bionomial(DR_{R-UK(age)y,i}, \Pr_{vCJD-DR-UK(age)y,i}) \quad \text{(IV.C.1.a-29)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\ y}$ - Total number of recovered plasma donors potentially infected with vCJD in year y

$$DR_{vCJD-R-UKy} = \sum_{Age=18-19 i=1day-3months}^{50-54} \sum^{>=5years} DR_{vCJD-R-UK(age)y,i} \quad \text{(IV.C.1.a-30)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Defy}$- Number of recovered plasma donors potentially infected with vCJD in year y and deferred by current policy

$$DR_{vCJD-R-UK-Defy} = \sum_{Age=18-19 i=3-5months}^{50-54} \sum^{>=5years} DR_{vCJD-R-UK(age)y,i} \quad \text{(IV.C.1.a-31)}$$

**Variable:** $DR_{vCJDS\text{-}UK\text{-}Resy}$ - Residual risk due to the number of recovered plasma donors potentially infected with vCJD not deferred by current policy and represented by the equation:

$$DR_{vCJD-R-UK-resy} = \sum_{Age=18-19}^{50-54} DR_{vCJD-R-UK(age)y,i=1day-3months} \quad \text{(IV.C.1.a-32)}$$

## A-IV. C. 1. a. v. d. Number of US recovered plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

As discussed in the sections above the most critical determinant in the model of whether exposure occurs is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (e.g., was prionemic) at the time of donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially have infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual will have vCJD agent in blood and plasma (prionemic) in year 2002

**Variable:** $DR_{vCJD\text{-}R\text{-}UKy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK (calculated in A-IV. C. 1. a. v. b)

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and in the last half incubation period of the disease.

$$DR_{vCJD-R-UK-LHy} = Binomial(DR_{vCJD-R-UKy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-33)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and met deferral criteria (calculated in A-IV. C. 1. a. v. c)

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}def\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who met deferral criteria.

$$DR_{vCJD-R-UK-Def-LHy} = Binomial(DR_{vCJD-R-UK-Defy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-34)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Resy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and did not meet deferral criteria (calculated in A-IV. C. 1. a. v. c).

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who did not meet deferral criteria

$$DR_{vCJD-R-UK-Res-LHy} = Binomial(DR_{vCJD-R-UK-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-35)}$$

## A-IV. C. 1. a. v. e. Number of all US plasma donors with a history of travel to the UK and potentially infected with vCJD

This section sums the total number of all US plasma donors, predicted by the model to donate to plasma pools used in manufacturing pdFVIII made from plasma collected in the US. This includes Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD and arrives at an estimate of the total number of US donors potentially infected with vCJD.

**Variable:** $DR_{vCJD\text{-}UK}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK

$$DR_{vCJD-UK} = \sum_{y=1980}^{1996} DR_{vCJD-S-UKy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UKy} \quad \text{(IV.C.1.a-36)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Def}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and met deferral criteria

$$DR_{vCJD-UK-Def} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Defy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Defy} \quad \text{(IV.C.1.a-37)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Res}$- Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and did not meet deferral criteria

$$DR_{vCJD-UK-Res} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Resy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Resy} \quad \text{(IV.C.1.a-38)}$$

## A-IV. C. 1. a. v. f. Total of all US plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

Again, whether a donor contains vCJD agent in their blood is a pivotal calculation for the model since a donation from such an individual would contain vCJD agent that may find its way into a large plasma pool of thousands of donations that are used to manufacture pdFVIII. This section sums the number of US Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD

and contain vCJD agent in their blood and arrives at an estimate of the total number of US donors potentially infected with vCJD and prionemic.

**Variable:** $DR_{vCJD\text{-}UK\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease

$$DR_{vCJD-UK-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-LHy} \quad \text{(IV.C.1.a-39)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Def\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease and met deferral criteria

$$DR_{vCJD-UK-Def-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Def-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Def-LHy} \quad \text{(IV.C.1.a-40)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Res\text{-}LH}$- Total number of plasma donors in the last half incubation period of the disease and did not meet deferral criteria

$$DR_{vCJD-UK-Res-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Res-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Res-LHy}$$

## A-IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

## A-IV.C.1.b.i. US plasma donors with a history of travel to France: Percentage of donors and travel duration

In this portion, blood donors are characterized by frequency and duration of travel to France since 1980. The risk of vCJD infection is a function of exposure to the BSE agent and is assumed to be proportional to the amount of time spent, or duration of travel, in France since 1980. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 5 years) in France since 1980, and to derive the frequencies for various durations of travel for 5 years or more. Since the baseline year to estimate potential vCJD risk for US donors in our model was 2002 trends in the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) were extrapolated from the year 1997 to 2002 when necessary to estimate potential travel characteristics and risk beyond 1996. The period of 5 years or more corresponds to the length of time in the current policy that defers donors who traveled to or resided in France. The travel survey data on blood donors pose a limitation because the survey was conducted on whole blood donors and may not exactly reflect the travel histories of plasma donors. Unfortunately, to our knowledge there is not travel data available on plasma donors. Therefore, we assumed that plasma donor travel characteristics to the France since 1980 are similar to those of whole blood donors and used this information in the FDA risk assessment. Some may argue that plasma donors travel less frequently than their blood donor counterparts so use of data on blood donors may overestimate the risk.

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC 2000).

**Variable:** $i$ - The duration interval used to group donors who had traveled to France from 1980-1996 based on the quantity of time spent in France during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (months) for interval $i$ representing the duration of travel or residence by US donors in France during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}FRi}$ – The cumulative percentage of blood donors who traveled to France within duration interval $i$ or longer.

**Data used in the model:** Travel data for US blood donors was obtained from a blood donor survey conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC, 2000).

**Variable:** $Perc_{BIDR\text{-}FRi}$ - Percentage of blood donors who have traveled to France within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}FRi}$

**Variable:** $Perc_{BIDR\text{-}FRi/FR}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France is represented by the equation:

$$Perc_{BIDR-FRi/FR} = (Perc_{BIDR-FRi} / CumPerc_{BIDR-FR,i>1day-1month}) \times 100\% \qquad \text{(IV.C.1.b-1)}$$

## IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

### A-IV.C.1.b.ii. Number of US plasma donors with a history of travel to France by age group, year of travel and duration of travel

This part of risk assessment calculates the annual number of US source and recovered plasma donors that traveled to France by specific year(s) and for a specific duration of time since 1980 by age. For the purposes of our analyses we grouped all donors and donors who traveled to France since 1980 into age groups of five year increments (20 – 24yrs, 25 – 29 yrs, etc) and the two year cohort for 18 and 19 years of age.

We estimated the frequency of donor travel by age group based on travel data for the US population and donor survey data. First, the total number of blood donors who traveled to France between 1980 and 1996 was estimated by multiplying the total number of US blood donors in a specific year by the percentage of donors (from survey data) who traveled to France. The percentage of donors in each age group that traveled to France since 1980 was calculated based on the total annual number donors who traveled to France since 1980 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel. Finally, the information on the number of donors from each age group who traveled was

calculated by year and duration of travel. The risk of a US donor acquiring vCJD is a function of duration of the stay, as well, the year(s) (since 1980) they resided in France.

- **Calculation of the annual number of blood donors who traveled to France from 1980 through 1996**

**Variable :** $DR_{BI}$-The annual total number of potential blood donors in the US

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR\text{-}FR}$ - The total percentage of US blood donors who traveled to France during the period from 1980 through 1996.

**Data used in the model:** Approximately, 15.6% of US blood donors reported a history of travel to France during the period from 1980 through 1996, based on the travel data of US blood donors (TSEAC, 2000).

**Variable:** $DR_{BI\text{-}FR}$ -Total number of blood donors who have traveled to France during 1980 and 1996

$$DR_{BI-FR} = DR_{BI} \times Perc_{BIDR-FR} \qquad \text{(IV.C.1.b-2)}$$

Then, the percentage donors in each age group that traveled to France between 1980 and 1996 was calculated based on the total number donors who traveled to France, the number of donors from each age group and the odds ratio of travel for each age group.

**Variable:** $DR_{BI(age)}$- Annual number of blood donors from each age group (calculated A-IV.C.1.a)

**Variable:** $Perc_{BIDR\text{-}FR(age)}$ - The percentage of US blood donors of an age group who traveled to France during the period from 1980 through 1996.

Total number of blood donor who traveled to France equals to the sum of donors from all age groups who have ever traveled to France:

$$DR_{BI-FR} = \sum_{age=18-19}^{65-69} (Perc_{BIDR-FR(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.b-3)}$$

**Variable:** $Odd_{T(age)}$- Odds ratios of each age group for the likelihood of travel compared to the group of age 18-19 year-olds.

**Data used in the model:** The odds ratio of travel for each age group was derived from the travel data obtained from 1980-1996 blood donor travel survey (TSEAC 2000). An odds ratio of 1 was assigned to donor group of age 18-19. Odds ratio of other age group represents the frequency of travel of those age groups compared to the group of age 18-19

$$Perc_{BlDR-FR(age)} = Odd_{T(age)} \times Perc_{BlDR-FR(18-19)} \quad \text{(IV.C.1.b-4)}$$

Replacing equation IV.C.1.b-4 for $Perc_{BlDR-FR(age)}$ in equation IV.C.1.b-3, percentage blood donors from age group of 18-19 who have traveled to France can be calculated by following equation:

$$Perc_{BlDR-FR(18-19)} = DR_{Bl-FR} / \sum_{age=18-19}^{65-69}(Odd_{T(age)} \times DR_{Bl(age)}) \quad \text{(IV.C.1.b-5)}$$

Then, the percentage of blood donors from other age groups who have traveled to France can be calculated using equation IV.C.1.b-4 (above).

## A-IV. C. 1. b. ii. a. Number of US Source Plasma donors with a history of travel to France by age group and specific year of travel

This section of the model computes the number of US Source Plasma donors who traveled to France based on annual total of Source Plasma donors and the estimated percentage of Source Plasma donors who have traveled to France between 1980 and 1996. Travel after 1996 was extrapolated from the percentage donors who travel between 1980 and 1996 and yearly travel data. To factor in travel frequency associated with donor age, the number of donors who have traveled was calculated by age groups.

**Variable:** ***age*** - Age of US plasma donors in groups of 5-year increments (20 – 24 yrs, 25 – 29yrs, etc) and the 18-19 year old cohort.

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B.1.b.) - the annual Source Plasma donations by age groups.

**Variable:** $Perc_{BlDR-FR(age)}$ (calculated in section A-IV.C.1.b.i..b.) - the percentage blood donors from an age group who have traveled to France between 1980-1996

**Variable:** $\dot{D}R_{S-FR(age)}$ - Number of Source Plasma donors who traveled to France from 1980 through 1996 by age group.

**Assumptions used in the model:** The percentage of Source Plasma donors who traveled to France is the same as blood donors of the same age group.

The number of Source Plasma donors who traveled to France from 1980 to 1996 by age group is represented by:

$$DR_{S-FR(age)} = DR_{S(age)} \times Perc_{BlDR-FR} \quad \text{(IV.C.1.b-6)}$$

The model assumes that the risk that a traveler was exposed to the BSE agent in France is associated with the magnitude of the BSE epidemic in the UK in the year of travel. Major exposure risk in France was presumably from consumption of beef imported from the UK that was produced from cattle infected with BSE. The model breaks down Source Plasma donors by year of travel, in order to estimate the potential

US donor vCJD risk more accurately by incorporating the information about the magnitude of the BSE epidemic in the UK in a given year.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV. C. 1. a. ii. a.).

**Variable:** $DR_{S\text{-}FR(age),y}$ - Number of Source Plasma donors with history of travel to France in year $y$ by age groups

The number of Source Plasma donors who have traveled to France was allocated to individual travel year based on the yearly distribution of visits to France.

$$DR_{S-FR(age)y} = DR_{S-FR(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.b-7)}$$

for travel during 1980-1996;

$$DR_{S-FR(age)y} = DR_{S-FR(age)1996} \times V_{y/1996} \qquad \text{(IV.C.1.b-8)}$$

for travel after 1996

**Assumption used in the model:** The yearly frequency and distribution of travel to France by US donors was ascertained from UK travel data and the blood donor survey data (TSEAC 2000). Therefore, in calculating the US donor risk for vCJD, donors the yearly distribution of travel visits by age groups was adjusted to account for the requirement that donors be 18 years of age or older. The model also accounted for the fact that some younger donors born during the period 1980 to 1986 may not have been born and would have essentially a zero chance of being exposed to BSE agent. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. b. ii. b. Number of US Source Plasma donors and duration of travel to France by age group

There are no data detailing the travel histories of Source Plasma donors available. Travel data for blood donors was used for Source Plasma donors after an adjustment for the frequency of travel based on the age of Source Plasma donors and the age-specific odds ratios for travel, which were obtained from 1980-1996 Blood Donor Travel Survey (TSEAC, 2000).

The model further subdivides the number of Source Plasma donors who traveled to France in a specific year by duration of stay. While there were no specific travel data detailing travel patterns to France since 1980 available for US Source Plasma donors, data on travel patterns for whole blood donors was used as a proxy. Donor travel in the model was subdivided into categories based on the percentage of blood donors

who traveled to France for certain durations (Watanabe, 2000) (>5yrs, ≤5yrs, etc) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to France from 1980-1996 based on the time they spent in France (same variable used above in section A-IV. C. 1. a. i.).

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1. i.).

**Variable:** $DR_{S\text{-}FR(age)y}$ - the number of Source Plasma donors who traveled to France in year $y$ by age group (calculated in A-IV. C. 1. b. ii. )

**Variable:** $Perc_{BIDR\text{-}FRi/FR}$- The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France (calculated in A-IV. C. 1. b. i. )

**Variable:** $DR_{S\text{-}FR(age)y,i}$ - Number of Source Plasma donors among an age group who have traveled to France in year $y$ for a duration of $i$ and is represented by the equation:

$$DR_{S-FR(age)y,i} = DR_{S-FR(age)y} \times Perc_{DR-FRi/FR} \qquad \text{(IV.C.1.b-9)}$$

## A-IV. C. 1. b. ii. c. Number of US recovered plasma donors with history of travel to France in a specific year between 1980 and 1996 – and by age group

Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year – and on average a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable:** $y$ – **Year of travel** (same variable used above in section A-IV. C. 1. b. ii. a) to France since 1980 by US plasma donors.

**Variable:** age – Age of the population by five-year increments (same variable used above in section A-IV. B. 1.).

**Variable:** $DR_{R(age)}$ - Number of potential recovered plasma donors per year by age group (described in section A-IV. B. 2).

**Variable:** $Perc_{BIDR\text{-}FR(age)}$ (calculated in section A-IV.C.1.b.i.)- The percentage blood donors from an age group who have traveled to France between 1980-1996

**Variable:** $DR_{R\text{-}FR(age)}$ - Number of recovered plasma donors who traveled to France from 1980 through 1996 by age groups and is represented by the equation:

$$DR_{R-FR(age)} = DR_{R(age)} \times Perc_{B|DR-FR(age)} \quad \text{(IV.C.1.b-10)}$$

The risk a traveler was exposed to BSE in France is proportional to the magnitude of the BSE epidemic in the UK in the year of travel. Because the major exposure risk in France is assumed to be from consumption of BSE contaminated beef imported from the UK , the model subdivides recovered plasma donors and groups them by year of travel. This provides a more precise estimate of the risk by incorporating the specific information on donor and donation characteristics and details that better capture the dynamics of the BSE epidemic in the UK on a year by year basis.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a)

**Variable:** $DR_{R\text{-}FR(age),y}$ - Number of recovered plasma donors with a history of travel to France in year $y$ by age groups

Number of recovered plasma donors who have traveled to France was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers.

$$DR_{R-FR(age)y} = DR_{R-FR(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \quad \text{(IV.C.1.b-11)}$$

for travel during 1980-1996;

$$DR_{R-FR(age)y} = DR_{R-FR(age)1996} \times V_{y/1996} \quad \text{(IV.C.1.b-12)}$$

for travel after 1996

**Assumptions used in the model:** The yearly distribution of travel to France by US donors is similar to the yearly distribution of travel to the UK by US donors- this is based on travel data and the blood donor survey (TSEAC 2000). The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. b. ii. d. US recovered plasma donors with a history of travel to France and specific year of travel and duration of travel by age group

Recovered plasma donors who traveled to France in a specific year ($DR_{R\text{-}FR(age)y}$) since 1980 were further partitioned into the subgroups based on travel duration and by 5-year age groups. Data on the percentage

of blood donors who traveled to France since 1980 for certain duration(s) (TSEAC, 2000) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to France from 1980-1996 based on the time they spent in France (same variable used above in section A-IV. C. 1.)

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R\text{-}FR(age),y}$ - The number of recovered plasma donors who traveled to France in year $y$ by age group (calculated in A-IV. C. 1. b. iv. c.)

**Variable:** $Perc_{BIDR\text{-}FRi/FR}$- The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France (calculated in A-IV. C. 1. b. ii. a.)

**Variable:** $DR_{R\text{-}FR(age)y,i}$- Number of recovered plasma donors among an age group who have traveled to France in year, $y$ ,for duration of $i$.

$$DR_{R-FR(age)y,i} = DR_{R-FR(age)y} \times Perc_{DR-FRi/FR} \qquad \text{(IV.C.1.b-13)}$$

## A-IV.C. 1. b. iii. US plasma donors with history of travel to France: Adjustment of the relative risk for France based on the magnitude of the UK BSE risk and by travel duration in a specific year

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because exposure to BSE in the UK was greater than any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence which is difficult to estimate. The relative risk value for France is 0:05 (or 5% of the UK risk). The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (TSEAC 2004). France received meat and bone meal from the UK during the BSE epidemic and approximately 5% of its beef was imported from the UK as of August 2006, France reported 20 cases of vCJD in its human population. Additionally, the model included calculations on the estimated duration of travel in France or residence by US plasma donors to generate a more accurate vCJD risk estimate. Current US vCJD geographic deferral policy defers donors with a history of residence in France for a period of 5 years or more since 1980.

## A-IV. C. 1. b. iii. a. US plasma donors with a history of travel to France: Average accumulated risk of residence since 1980

**Variable:** $R_{FR}$ – The cumulative risk of individual residents of France from 1980 till present; assuming that the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The average cumulative risk of a resident of France since 1980 is 0.05 relative to 1, the average accumulated risk of UK individual since 1980, based on UK beef imports, vCJD cases and indigenous BSE in France

**A-IV. C. 1. b. iii. b. US plasma donors with history of travel to France: Proportional risk of individual resident per year since 1980**

**Variable:** $y$ – **year** (same variable used in A-IV. C. 1. a. iv.)

**Variable:** $BSE_{UKy}$ (same variable used in A-IV. C. 1. a. iii.)

**Variable:** $BSE_{FRy}$ - Annual numbers of reported BSE cases in France including indigenous and imported cases.

**Data used in the model:** Data on the annual number of reported BSE cases in France was obtained from the World organization for animal health (OIE) (2005).

**Variable:** $R_{FRy}$ - Proportional risk in France in a specific year

**Assumptions used in the model:**

- Variant CJD Risk in France occurred starting in 1980 to the present. Evidence indicates that vCJD and BSE cases are still emerging.
- Risk is additive, and can be pro-rated in a yearly and further monthly basis.
- Yearly rate of the risk in France is proportional to the reported BSE annual cases (including indigenous and imported cases) in France.

## A-IV. C. 1. b. iii. c. US plasma donors with a history of travel to France: Potential vCJD risk for donors who traveled in year y for a period of i

**Variable:** $R_{DR\text{-}FR,i}$ - The risk of an individual US donor who traveled to France in a specific year with a specific duration of travel, assuming a cumulative risk for a UK individual from 1980 through 1996 is 1 (or equivalent to the UK vCJD prevalence for the entire UK population).

The vCJD risk for the US plasma donor subpopulation that traveled to France in a specific year for a specific duration was calculated by applying a prorated monthly rate of risk, which was calculated based on the yearly rate of the risk in France during the year of travel. The blood donor travel survey (TSEAC, 2000) collected information on the accumulated stay of the donors in France from 1980 through 1996, which, for simplicity, was used to calculate the duration of consecutive stay, when calculated the risk.

**Assumptions used in the model:**

- Risk is proportional to the duration of the stay
- All travelers have consecutive stays
- US plasma donor subpopulation having more than 5 years accumulated stay in France have average risk of 0.05, which is the same as the average risk of individual resident of France (equivalent to 5% of the UK vCJD prevalence).

$$R_{DR-FRy,i} = (R_{FRy}/12) \times D_i \qquad \text{(IV.C. 1. b•-14)}$$

for $i$< 1 years;

$$R_{DR-FRy,i} = (Average(R_{FRy} : R_{FR(y+i)})/12) \times D_i \qquad \text{(IV.C.1.b-15)}$$

for 5 years <i>=1 year;

$$R_{DR-FRy,i} = 0.05 \qquad \text{(IV.C.1.b-16)}$$

for $i$>= 5 years

## A-IV.C. 1. b. iv. US plasma donors with history of travel to France: Probability of vCJD infection for donor of a specific age group, in a specific year, for a specific duration, i

This section describes the portion of the model that estimates the potential probability that a US plasma donor in a specific age group who traveled to France for a specific duration since 1980 was infected with vCJD.

**Variable:** $Pr_{vCJD-FR(age)}$ Probability of vCJD infection in an individual resident of France in a specific age.

**Variable:** $Pr_{vCJD-DR-FR(age)y,i}$ - Probability of infection for individual US plasma donor of a specific age group who have traveled to France in a specific year for a specific duration.

**Assumption about variable:** Probability of vCJD infection being proportional to the risk of exposure to the BSE agent and represented by the equation:

$$\Pr_{vCJD-DR-FR(age)y,i} = \Pr_{vCJD-UK(age)} \times R_{DR-FRy,i} \qquad \text{(IV.C.1.b-17)}$$

## A-IV.C. 1. b. v. Total number of all US plasma donors with a history of travel to France: Number potentially infected with vCJD

## A-IV.C. 1. b. v. a. Number of US Source Plasma donors with a history of travel to France and potentially infected with vCJD

Plasma is collected from Source Plasma donors in a process called plasmapheresis in which approximately 700 milliliters of plasma are collected. Source Plasma donors donate an average of 14 units per year, but can donate up to 48 times per year.

This component of the model estimates the number of US Source Plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}FR(age)y,i}$ - Number of Source Plasma donors potentially infected with vCJD during travel to France since 1980 by age, year and duration of travel.

$$DR_{vCJD-S-FR(age)y,i} = Bionomial(DR_{S-FR(age)y,i}, \Pr_{vCJD-DR-FR(age)y,i}) \quad \text{(IV.C.1.b-18)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FRy}$ - Number of Source Plasma donors potentially infected with vCJD in year y during travel/residence in France.

$$DR_{vCJD-S-FRy} = \sum_{Age=18-19yrs}^{50-54yrs} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-S-FR(age)y,i} \quad \text{(IV.C.1.a-19)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Defy}$ - Number of Source Plasma donors potentially infected with vCJD in year y and meet deferral criteria.

Current deferral policy defers individuals who have a history of travel to France since 1980 for an accumulated time of over 5 years from donating blood and plasma. The number of potentially infected donors who meet deferral criteria was calculated by equation:

$$DR_{vCJD-S-FR-Defy} = \sum_{Age=18-19}^{50-54yrs} DR_{vCJD-S-FR(age)y,i>=5years} \quad \text{(IV.C.1.a-20)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Resy}$ - Residual risk due to the number of Source Plasma donors potentially infected with vCJD in year y and not deferred by current policy

$$DR_{vCJD-S-FR-Resy} = \sum_{Age=18-19yrs}^{50-54yrs} \sum_{i=1-30days}^{>3-5years} DR_{vCJD-S-FR(age)y,i} \quad \text{(IV.C.1.a-21)}$$

## A-IV. C. 1. b. v. b. Number of US Source Plasma donors with history of travel to France and potentially infected and vCJD agent is present in the blood

Perhaps the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (i.e., was prionemic at the time of donation). Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of Source Plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** *y*-The calendar year in which a plasma donor traveled and infected with vCJD

**Assumption used in the model:** This risk assessment assesses the risk for pdFVIII product made in 2002 (but risk is assumed to be similar up to the year 2006).

**Variable:** $T_{Inf\text{-}2002y}$-Time Period between infection/travel and year of 2002 when the plasma was collected

**Variable:** $Pr\text{-}LH_y$-Probability the individual is in the last half incubation period of the disease, if infected in year y

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection and travel and 2002 when the plasma was collected

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.b-22)}$$

For an individual to have vCJD agent present in their blood and plasma (prionemic) in 2002, the elapsed period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or less than the remaining half of incubation period of the disease; in another words, the incubation period of the disease should be equal to or less than twice as much as $I_{Inf\text{-}2002y}$.

**Assumption used in the model** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially, in this case infection, incubation period of the disease, etc. The distribution is defined by two parameters (or arguments) that produce the shape of the curve and generates a mean incubation period of 14 years and a median incubation period of 13 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual will be prionemic in the year 2002, was determined using the distribution:

Cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

Variable: $DR_{vCJD\text{-}S\text{-}FR_y}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France (calculated in A-IV. C. 1. b. v. a.)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and in the last half incubation period of the disease.

$$DR_{vCJD-S-FR-LHy} = Binomial(DR_{vCJD-S-FRy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.b-23)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}defy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and met deferral criteria (calculated in A-IV. C. 1. b. v. a)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}def\text{-}LHy}$ - Total number of Source Plasma donors in the last-half of the incubation period of the disease who met deferral criteria.

$$DR_{vCJD-S-FR-Def-LHy} = Binomial(DR_{vCJD-S-FR-Defy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.b-24)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Resy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and did not met deferral criteria (calculated in A-IV. C. 1. b. v. a)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Res\text{-}LHy}$ - Total number of Source Plasma donors in the last half incubation period of the disease who did not met deferral criteria or were not deferred is represented by the equation:

$$DR_{vCJD-S-FR-Res-LHy} = Binomial(DR_{vCJD-S-FR-Resy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.b-25)}$$

## A-IV.C. 1. b. v. c. Number of US recovered plasma donors with a history of travel to France and potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}R\text{-}FR(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to France since 1980 by age, year and duration of travel

$$DR_{vCJD-R-FR(age)y,i} = Bionomial(DR_{R-FR(age)y,i}, \Pr_{vCJD-DR-FR(age)y,i}) \quad \text{(IV.C.1.b-26)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}FRy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y:

$$DR_{vCJD-R-FRy} = \sum_{Age=18-19}^{50-54} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.b-27)}$$

Current deferral policy defers individuals who have history of travel to France since 1980 for an accumulated residence of 5 years or more from donating blood and plasma. The number of potentially infected donors who meet the deferral criteria was calculated by equation:

$$DR_{vCJD-R-FR-Defy} = \sum_{Age=18-19}^{50-54\,yrs} DR_{vCJD-R-FR(age)y,i>=5\,years} \quad \text{(IV.C.1.b-28)}$$

**Variable:** $DR_{vCJD-S-FR-Resy}$- The residual risk due to the number of recovered plasma donors potentially infected with vCJD in year y and not deferred by current policy

$$DR_{vCJD-R-FR-Resy} = \sum_{Age=18-19\,yrs}^{50-54\,yrs} \sum_{t=1-30\,days}^{>3-5\,years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.b-29)}$$

## A-IV. C. 1. b. v. d. Number of US recovered plasma donors with history of travel to France and potentially infected and vCJD agent is present in the blood

As discussed in the sections on Source Plasma (above) the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (i.e., was prionemic at the time of donation). Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH-y}$ -The probability an individual will have vCJD agent present in their blood or present (prionemic) at the time of donation in the year 2002 (calculated in A-IV.C.1.a.v. b.)

Variable: $DR_{vCJD-R-FRy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in France (calculated in A-IV. C. 1. b. v. c.)

**Variable:** $DR_{vCJD-R-FR-LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in France and in the last half incubation period of the disease.

$$DR_{vCJD-R-FR-LHy} = Binomial(DR_{vCJD-R-FRy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.b-30)}$$

**Variable:** $DR_{vCJD-R-FR-defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in France and met deferral criteria (calculated in A-IV. C. 1. b. v. c)

**Variable:** $DR_{vCJD-R-FR-def-LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who met deferral criteria and presumably were deferred from donation.

$$DR_{vCJD-R-FR-Def-LHy} = Binomial(DR_{vCJD-R-FR-Defy,}\ \Pr_{LH-y}) \quad \text{(IV.C.1.b-31)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}FR\text{-}Resy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in France and did not met deferral criteria and were likely not deferred from donation (calculated in A-IV. C. 1. b. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}FR\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who did not meet deferral criteria and were likely not deferred from donation.

$$DR_{vCJD-R-FR-Res-LHy} = Binomial(DR_{vCJD-R-FR-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.b-32)}$$

## A-IV. C. 1. b. v. e. Number of all US plasma donors with history of travel to France and potentially infected with vCJD

This section sums the number of all US plasma donors, predicted by the model to donate to plasma pools used in manufacturing pdFVIII made from plasma collected in the US. This includes recovered plasma donors and Source Plasma donors, and generates an estimate for the total number of donors potentially infected with vCJD during extended travel to France since 1980.

**Variable:** $DR_{vCJD\text{-}FR}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in France

$$DR_{vCJD-FR} = \sum_{y=1980}^{1996} DR_{vCJD-S-FRy} + \sum_{y=1980}^{1996} DR_{vCJD-R-FRy} \quad \text{(IV.C.1.b-33)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Def}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in France and meet deferral criteria

$$DR_{vCJD-FR-Def} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Defy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Defy} \quad \text{(IV.C.1.b-34)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Res}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and did not meet deferral criteria

$$DR_{vCJD-FR-Res} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Resy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Resy} \quad \text{(IV.C.1.b-35)}$$

## A-IV. C. 1. b. v. f. Total number of US plasma donors with history of travel to France and are potentially infected and vCJD agent is present in the blood

Again, whether a donor contains vCJD agent in their blood is a pivotal calculation in the model since a donation from such an individual would contain vCJD agent that may find its way into a large plasma pool of thousands of donations that are used to manufacture pdFVIII. This section sums the number of

US Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD and contain vCJD agent in their blood and arrives at an estimate of the total number of US donors potentially infected with vCJD and who are prionemic.

**Variable:** $DR_{vCJD\text{-}FR\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease

$$DR_{vCJD-FR-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-S-FR-LHy} \quad \text{(IV.C.1.b-36)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Def\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease and met deferral criteria

$$DR_{vCJD-FR-Def-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Def-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Def-LHy} \quad \text{(IV.C.1.b-37)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Res\text{-}LH}$- Total number of plasma donors in the last half incubation period of the disease and did not met deferral criteria

$$DR_{vCJD-FR-Res-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Res-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Res-LHy} \quad \text{(IV.C.1.b-38)}$$

## A-IV.C. 1. c. Number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) potentially infected and vCJD agent is present in the blood

## A-IV.C.1.c.i. US recovered plasma donors with a history of travel to countries in Europe: Percentage of US donors and travel duration

In this portion of the FDA risk assessment, US blood (recovered plasma) donors are characterized by frequency and duration of travel to countries in Europe (other than the UK) during the period 1980-1996. The risk of vCJD infection is a function of exposure to the BSE agent and is assumed to be proportional to the amount of time spent, or duration of travel, in countries in Europe (other than the UK) since 1980. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 5 years) in other countries in Europe (other than the UK and France) since 1980, and to derive the frequencies for various durations of travel for 5 years or more. The period of 5 years or more corresponds to the length of time in the current policy that defers blood (recovered plasma) donors who traveled to or resided in European countries (other than the UK).

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the TSEAC in 2000.

**Variable:** $i$ - The duration interval used to group donors who had traveled to countries in Europe from 1980-1996 based on the quantity of time spent in Europe (other than the UK and France) during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (in months) for interval $i$ representing the duration of travel or residence by US donors in countries in Europe (other than the UK and France) during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}EUi}$ – The cumulative percentage of blood donors who traveled to countries in Europe (other than the UK and France) within duration interval $i$ or longer.

**Data used in the model:** Travel data for US blood donors was obtained from a blood donor survey conducted by the American Red Cross and presented at the TSEAC in 2000.

**Variable:** $Perc_{BIDR\text{-}EUi}$ - Percentage of blood donors who traveled to countries in Europe (other than the UK and France) within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}EUi}$

**Variable:** $Perc_{BIDR\text{-}EUi/EU}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to countries in Europe (other than the UK and France) is represented by the equation:

$$Perc_{BIDR\text{-}EUi/EU} = (Perc_{BIDR\text{-}EUi} / CumPerc_{BIDR\text{-}EU,i>1day\text{-}1month}) \times 100\% \qquad \text{(IV.C.1.c-1)}$$

The following portion of the risk assessment estimated the frequency of travel for each recovered plasma donor by age group based on travel data of blood donors. First, estimates for blood donors who traveled to countries in Europe (other than the UK and France) between 1980 and 1996 was calculated to generate the total number of US blood donors and percentages of donors who traveled. For the purposes of our analyses we grouped all donors and donors who traveled to countries in Europe since 1980 into age groups of five-year increments (20 – 24yrs, 25 – 29 yrs, etc) and for the 18 – 19 year old cohort. The percentage of donors in each age group that traveled to countries in Europe since 1980 was calculated based on the total annual number donors who traveled to Europe since 1980 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel.

- **Calculation of the annual number of blood donors who traveled to countries in Europe (other than the UK and France) from 1980 through 1996**

**Variable :** $DR_{BI}$ - The annual total number of potential blood donors in the US.

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR\text{-}EU}$ - The total percentage of US blood donors who traveled to countries in Europe (other than the UK and France) during the period from 1980 through 1996.

**Data used in the model:** Approximately, 15.6% of US blood donors have histories of travel to countries in Europe (other than the UK and France) during the period from 1980 through 1996, based on the travel data of US blood donors (TSEAC, 2000).

**Variable:** $DR_{BI\text{-}EU}$ -Total number of blood donors who have traveled to countries in Europe (other than the UK and France) during the period 1980 - 1996.

$$DR_{BI-EU} = DR_{BI} \times Perc_{BIDR-EU} \qquad \text{(IV.C.1.c-2)}$$

Then, the percentage of donors in each age group who traveled to countries in Europe (other than the UK and France) between 1980 and 1996 was calculated based on the total number donors who traveled to countries in Europe, the number of donors from each age group, and the odds ratio of travel for each age group.

**Variable:** $DR_{BI(age)}$- Annual number of blood donors from each age group (calculated IV.C.1.a)

**Variable:** $Perc_{BIDR\text{-}EU(age)}$ - Annual percentage of US blood donors of an age group who traveled to countries in Europe during the period from 1980 through 1996.

Total number of blood donor who traveled to countries in Europe (other than the UK and France) equals to the sum of donors from all age groups who have ever traveled to Europe:

$$DR_{BI-EU} = \sum_{age=18-19}^{65-69} (Perc_{BIDR-EU(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.c-3)}$$

**Variable:** $Odd_{T(age)}$- The odds ratio for each five-year age group (e.g. 20-24 yrs, etc.) of travelers was compared to the group of age 18-19 yr olds.

**Data used in the model:** The odds ratios of travel to countries in Europe (other than the UK and France) for each age group was derived from the travel data obtained from 1980-1996 blood donor travel survey. An odds ratio of 1 was assigned to the donor group of age 18-19 yr olds. The odds ratios for the other age groups were generated by comparing the frequency of travel for those age groups to the group of age 18-19 yr-olds.

$$Perc_{BIDR-EU(age)} = Odd_{T(age)} \times Perc_{BIDR-EU(18-19)} \qquad \text{(IV.C.1.c-4)}$$

The percentage of blood donors from the age group of 18-19 yr olds who have traveled to countries in Europe (other than the UK and France) can be calculated by the following equation:

$$Perc_{BIDR-EU(18-19)} = DR_{BI-EU} / \sum_{age=18-19}^{65-69} (Odd_{T(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.c-5)}$$

Then, the percentage blood donors from other age groups who traveled to Europe (other than the UK and France) can be calculated using the equation (A-IV.C.1.c-4).

## A-IV. C. 1. c. ii. US recovered plasma donors who traveled to countries in Europe: Total number by year of travel, duration of travel and by age group

This part of the risk assessment calculates the annual number of US recovered plasma donors who traveled to countries in Europe (other than the UK and France) since 1980. The number of donors from an age group who traveled was calculated by year and duration of travel. The risk of a US donor acquiring vCJD is a function of duration of the stay, as well as the year(s) (since 1980) they resided in Europe.

## A-IV. C. 1. c. ii.a. Number of US recovered plasma donors who traveled to Europe in a specific year between 1980 and 1996 by age group

This portion of the model estimates the potential vCJD risk for US recovered plasma donors with a history of travel to countries in Europe (other than the UK and France). Recovered plasma is derived from single units of Whole Blood as a by-product in the preparation of blood components from whole blood collection and is intended for further manufacturing. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year – and on average a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable:** $y$ – year of travel (same variable used above in section A-IV. C. 1. b. ii. a.) to a country in Europe (other than the UK or France) since 1980 by US plasma donors.

**Variable: age** – Age groups of the population by five-year increments (same variable used above in section A-IV. B. 1.) and the two year cohort for 18 and 19 year olds for US recovered plasma donors who traveled to countries in Europe (other than the UK and France).

**Variable:** $DR_{R(age)}$ - Number of potential US recovered plasma donors per year by age group (described in section A-IV. B. 2.).

**Variable:** $Perc_{BIDR\text{-}EU(age)}$ (calculated in section A-IV.C.1.b.ii.b.)- The percentage US blood donors by age group who have traveled to countries in Europe (other than the UK and France) between 1980-1996.

**Variable:** $DR_{R\text{-}EU(age)}$ - Number of recovered plasma donors who traveled to countries in Europe (other than the UK and France). from 1980 through 1996 by age group and is represented by the equation:

$$DR_{R-EU(age)} = DR_{R(age)} \times Perc_{BIDR-EU(age)} \qquad \text{(IV.C.1.c-10)}$$

The risk a traveler was exposed to BSE in Europe is proportional to the magnitude of the BSE epidemic in the UK in the year of travel. Because the major exposure risk in Europe was assumed to be from consumption of beef contaminated with BSE agent imported from the UK. The model groups recovered plasma donors by year of travel. This provides a more precise estimate of the risk by incorporating the (age and year) specific information and details that better capture the dynamics of the BSE epidemic in the UK on a year by year basis. Travel for the years not covered by the UK National Survey 1997 to 2002 relied on extrapolation of trends from 1996 and before to estimate travel characteristics up to the baseline year of 2002 in the model (see equation A-IV.C.1.c-12 (below)).

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a.)

**Variable:** $DR_{R\text{-}EU(age),y}$ - Number of recovered plasma donors who traveled to Europe in year $y$ by age groups.

The number of recovered plasma donors who have traveled to countries in Europe (other than the UK and France) was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers.

$$DR_{R\text{-}EU(age)y} = DR_{R\text{-}EU(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.c-11)}$$

for travel during 1980-1996;

$$DR_{R\text{-}EU(age)y} = DR_{R} \times V_{y/1996} \qquad \text{(IV.C.1.c-12)}$$

for travel after 1996.

**Assumption used in the model:** The yearly distribution of travel to countries in Europe (other than the UK and France) by US recovered plasma donors is similar to the yearly distribution of travel to the UK by US travelers. The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. c. ii. b. Number of US recovered plasma donors who traveled to countries in Europe by specific year of travel and duration of travel by age group

Recovered plasma donors who traveled to countries in Europe (other than the UK and France) in a specific year ($DR_{R\text{-}EU(age)y}$) since 1980 were further partitioned into the subgroups in the model based on travel duration and by 5-year age groups and also the two year cohort of donors 18 and 19 years of age. Data on the percentage of blood donors who traveled to countries in Europe (other than the UK and France) since 1980 for a certain duration(s) (TSEAC, 2000) were used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to countries in Europe (other than the UK and France) from 1980-1996 based on the time spent (same variable used above in section A-IV. C. 1.).

**Variable:** $D_i$ - The average duration of time for interval *i* (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R\text{-}EU(age),y}$ - The number of recovered plasma donors who traveled to countries in Europe (other than the UK and France) in year *y* by age group

**Variable:** $Perc_{BIDR\text{-}EU/FEU}$- The percentage of blood donors who traveled for a specific duration interval *i* among all donors who have ever traveled to countries in Europe (other than the UK and France) (calculated in A-IV. C. 1. c. i).

**Variable:** $DR_{R\text{-}EU(age)y,i}$- Number of recovered plasma donors within a specific age group who have traveled to countries in Europe (other than the UK and France) in year, *y* ,for duration of *i*.

$$DR_{R-EU(age)y,i} = DR_{R-EU(age)y} \times Perc_{DR-EU/EU} \qquad \text{(IV.C.1.c-13)}$$

## A-IV.C. 1. c. iii. US recovered plasma donors who traveled to countries in Europe: Adjustment of relative risk for the proportion of exposure to the BSE agent per year and by duration of travel and by age group

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because exposure to BSE in the UK was greater than that of any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence, which is difficult to estimate. Again, based on information in FDA guidance (2002), the relative risk value for France was assumed to be 0.05 (or 5% of the UK risk). The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (FDA 2002). Countries in Europe (other than the UK and France) received meat and bone meal from the UK during the BSE epidemic and approximately 1.5% of their beef was imported from the UK. Additionally, the model included calculations on the estimated duration of travel or residence in Europe by US plasma donors to generate a more accurate vCJD risk estimate. Current US vCJD geographic deferral policy defers blood donors with a history of residence in countries in Europe (other than the UK and France) for a period of 5 years or more since 1980; this policy does not include Source Plasma donors.

## A-IV. C. 1. c. iii. a. Number of US recovered plasma donors with a history of travel to countries in Europe: Average accumulated vCJD risk for donors since 1980, assuming that the average accumulated risk for a UK individual since 1980 is 1

This section calculates the number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) and their average accumulated vCJD risk since 1980, assuming that the average accumulated risk for a UK individual since 1980 is 1.

**Variable:** $R_{EU}$ - Ac– The cumulative vCJD risk of an individual resident of countries in Europe (other than the UK and France) from 1980 till now; assuming the accumulated risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The average accumulated vCJD risk for travel to countries in Europe (other than the UK and France) by an individual resident since 1980 was assumed to be 0.015 relative to 1, the average accumulated risk of UK individual since 1980, based on UK. It was assumed that approximately 1.5% or less of the beef imports, imported into many countries since 1980 was imported from the UK. The 0.015 relative risk value also considers the number of human vCJD cases present and the presence of indigenous BSE in European countries (other than the UK and France) since 1980.

**A-IV. C. 1. c. iii. b. US plasma donors with a history of travel to countries in Europe: Proportional risk per individual resident per year since 1980**

**Variable:** $Y_{epi}$ –*y* Years of BSE epidemic in countries in Europe (other than the UK and France).

**Variable:** $BSE_{UKy}$ Annual numbers of reported BSE cases in the UK.

**Variable:** $BSE_{EUy}$ - Annual numbers of reported BSE cases in European countries other than the UK and France.

**Data used in the model:** Data on the annual number of reported cases of BSE in cattle in European countries was obtained from the Orgainization Internationale de Epizootics (OIE) (2005).

**Variable:** $R_{EUy}$ - Proportional vCJD risk for European countries (other than the UK and France) in a specific year

**Assumptions used in the model:**

- The risk of vCJD and BSE in European countries (other than the UK and France) has been present since 1980 and continues to present day. As of August 2006, very few cases of BSE continue to be reported in European countries (other than the UK and France)..
- The vCJD risk is assumed to be additive, and can be prorated on a yearly or monthly basis.
- The yearly rate of the vCJD risk in European countries (other than the UK and France) is proportional to the reported BSE annual cases in Europe (including indigenous and imported cases)

## A-IV. C. 1. c. iii. b. US recovered plasma donors with a history of travel to countries in Europe: Potential vCJD risk for an individual in year y for a period of i since 1980

**Variable:** $R_{DR\text{-}EUy_{w}i}$ - Risk for individual US donors who traveled to countries in Europe other than the UK and France in a specific year for a specific duration, assuming the accumulative risk of a UK person resident in the years 1980 through 1996 is 1.

The vCJD risk for the US plasma donors with less than 5 years accumulated stay in Europe was calculated based on travel to European countries other than the UK and France in a specific year for a specific duration of travel. Potential exposure risk was calculated using a prorated monthly rate, which was calculated based on the yearly rate of the risk (1 month = 1/12 x yearly risk) in Europe during the year of travel. US plasma donors with a total accumulated stay in countries in Europe (other than the UK or France) of 5 years or more is assumed to have average risk of 0.015, which is the same as the risk of an individual citizen or long-term resident of a country in Europe (other than the UK or France). Information on duration of accumulated stays was collected in the blood donor travel survey; however, for simplicity we assumed all travel was consecutive. The blood donor travel survey (TSEAC 2000) collected information on the accumulated stay of US donors who stayed in Europe (other than the UK or France) from 1980 through 1996. For simplicity, these data were used to estimate the duration of consecutive stay, which was used to calculate the potential vCJD risk for recovered plasma donors.

Assumptions used in the model:

- Risk is proportional to the duration of the stay
- All the travelers' stays were assumed to be single and consecutive stays.
- US plasma donor subpopulation having 5 or more years accumulated stay in countries in Europe (other than the UK or France) have an average risk of 0.015, which is the same as the average risk of individual European resident.

For < 1 year;

$$R_{DR-EUy,i} = (Average(R_{EUy} : R_{EU(y+i)})/12) \times D_i \qquad \text{(IV. C. 1. c-14)}$$

for 5 years <$i$>=1 year;

$$R_{DR-EUy,i} = 0.015 \qquad \text{(IV. C. 1. c-15)}$$

for $i$>= 5 years

## A-IV. C. 1. c. iv. US recovered plasma donors with a history of travel to countries in Europe: Probability of vCJD infection for a donor of a specific age group, who traveled in a specific year for a specific duration, i

This section describes the portion of the model that estimates the potential probability that a US plasma donor in a specific age group, who traveled to countries in Europe (other than the UK or France) for a specific duration since 1980 was infected with vCJD.

**Variable:** $\mathbf{Pr_{vCJD\text{-}UK(age)}}$ – the probability of infection for an individual UK resident of a specific age group (calculated in A-IV. C. 1. a. iv.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}EU(age)y,i}$ - Probability of infection for an individual US plasma donor of a specific age group who have traveled to countries in Europe other than the UK and France in a specific year with specific duration.

Assumption used in the model: The probability of vCJD infection is proportional to the risk of exposure

$$\Pr_{vCJD-DR-EU(age)y,i} = \Pr_{vCJD-UK(age)} \times R_{DR-EUy,i} \quad \text{(IV.C.1.c-16)}$$

## A-IV.C. 1. c. v. Number of US recovered plasma donors with a history of travel to countries in Europe: Number potentially infected with vCJD.

**Variable:** $DR_{vCJD\text{-}R\text{-}EU(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to countries in Europe (other than the UK and France) since 1980 by age, year and duration of travel

$$DR_{vCJD-R-EU(age)y,i} = Bionomial(DR_{R-EU(age)y,i}, \Pr_{vCJD-DR-EU(age)y,i}) \quad \text{(IV.C.1.c-26)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EUy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y

$$DR_{vCJD-R-FRy} = \sum_{Age=18-19}^{50-54} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.c-27)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Defy}$- Number of recovered plasma donors potentially infected with vCJD in year y and deferred by current policy

Current FDA guidance (FDA 2002) recommends deferral of individuals who have history of travel to countries in Europe (other than the UK or France) since 1980 for an accumulated stay of 5 years or more from donating blood. The number of potential vCJD-infected recovered plasma donors who meet current deferral criteria (FDA 2002) was calculated by the equation:

$$DR_{vCJD-R-EU-Defy} = \sum_{Age=18-19}^{50-54} DR_{vCJD-R-EU(age)y,i>=5years} \quad \text{(IV.C.1.c-28)}$$

**Variable:** $DR_{vCJDS\text{-}EU\text{-}Resy}$ – The residual risk associated with recovered plasma donors potentially infected with vCJD that meet deferral criteria but because of limitations in the donor screening process and are not deferred by current policy; is represented by the equation:

$$DR_{vCJD-R-EU-Resy} = \sum_{Age=18-19}^{50-54} \sum_{i=1-30days}^{>=3-5years} DR_{vCJD-R-EU(age)y,i} \quad \text{(IV.C.1.c-29)}$$

## A-IV. C. 1. c. vi. Number of US recovered plasma donors who traveled to countries in Europe: Number potentially infected and vCJD agent is present in the blood

The most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor that contained infectious vCJD agent in their blood (or was prionemic) at the time of donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH-y}$ -The probability an individual was prionemic in year 2002.

Variable: $DR_{vCJD\text{-}R\text{-}EUy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in European countries (other than France).

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in a country in Europe (other than the UK or France) and are in the last half incubation period of the disease (and has vCJD agent present in their blood).

$$DR_{vCJD-R-EU-LHy} = Binomial(DR_{vCJD-R-EUy,}\ \Pr_{LH-y}) \qquad \text{(IV.C.1.c-30)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in European countries (other than the UK or France) and met deferral criteria and were presumably deferred from donation .

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}def\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease (and presumably has vCJD agent present in their blood) who met deferral criteria and were presumably deferred from donation.

$$DR_{vCJD-R-EU-Def-LHy} = Binomial(DR_{vCJD-R-EU-Defy,}\ \Pr_{LH-y}) \qquad \text{(IV.C.1.c-31)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Resy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in countries in Europe (other than the UK or France) and did not meet the deferral criteria and were likely not deferred .

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half of the incubation period of the disease who did not met the deferral criteria and were likely not deferred from donation:

$$DR_{vCJD-R-EU-Res-LHy} = Binomial(DR_{vCJD-R-EU-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.c-32)}$$

## A-IV. C. 1. d. Number of US plasma donors deployed by the military in the UK or other countries in Europe and potentially infected with vCJD

### A-IV. C. 1. d. i. Percentage of US plasma donors deployed at US military bases in the UK or other countries in Europe during the years 1980 through 1996

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of US blood donors who were military residents in countries in Europe for ≥ 6 months from 1980 through 1996.

Assumption used in the model: Approximately 2% of US blood donors have been military residents in European countries between 1980-1996 (TSEAC 2002). There were no data for plasma donors, therefore, data for US blood donors was used to estimate the number of US donors stationed in US military facilities during the period 1980-1996

- The FDA model assumed that the same percentage of plasma donors have been in the military and deployed in European countries as blood donors.

### A-IV. C. 1. d. ii. Number of US plasma donors deployed by Military in the UK or other countries in Europe by year of deployment since 1980

### A-IV. C. 1. d. ii. a. Number of US Source Plasma donors stationed at US military facilities in the UK or countries in Europe during the period from 1980 -1996 by year of deployment

**Variable:** ***y*** - Calendar year of deployment

**Variable:** ***DODy*** - Number of US military residents, their family and dependents who resided on US military facilities in Europe by year from 1980 through 1996.

**Assumption used in the model:** The risk of BSE exposure and vCJD infection for donors previously deployed to US military facilities in the UK or countries in Europe after 1996 was assumed to be negligible, because it is assumed that most of the risk was associated with imported UK beef. Food chain controls put in place in the UK after 1996 were assumed to reduce the BSE exposure risk to negligible levels (TSEAC, 2002) and shipment of UK beef to US military facilities had stopped in 1996 or earlier.

**Variable:** ***age*** - age of donors in grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group (same variable used above in section A-IV.C.1.a.ii.)

$$Perc_{DR-DODy} = (DOD_y / \sum_{y=1980}^{1996} DOD_y) \times 100\% \quad \text{(IV.C.1.d-1)}$$

**Variable:** $DR_{S(age)}$ – Age of donors of Source Plasma (calculated in section A-IV.B. 1.)

**Variable:** $Perc_{DR\text{-}DOD}$ – Percentage of Source Plasma donors who have a history of military deployment in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{S\text{-}DOD(age)}$ - Estimated annual number of Source Plasma donors who have history of military deployment in the UK or Europe by age

**Assumption about variable:** We assumed 3% of Source Plasma donors have a history of military deployment and residence in the UK, France or other countries of Europe during any of the years from 1980-1996 and would have similar donation demographics and characteristics as whole blood donors.

The estimated annual number of Source Plasma donors who have a history of military deployment in Europe by age is represented by the equation:

$$DR_{S-DOD(age)} = DR_{S(age)} \times Perc_{DR-DOD} \quad \text{(IV.C.1.d-2)}$$

**Variable:** $DR_{S\text{-}DOD(age)y}$ – Number estimated annual number of Source Plasma donors who have resided on military bases in Europe by age and deployment year

$$DR_{S-DOD(age)y} = DR_{S-DOD(age)} \times Perc_{DR-DODy} \quad \text{(IV.C.1.d-3)}$$

## A-IV. C. 1. d. ii. b. Number of recovered plasma donors with a history of military deployment at US military facilities in the UK or other countries in Europe during the period 1980-1996 by year of deployment

**Variable:** *age* - Age of donors grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group. No data for the yearly distribution of deployment of military plasma donors in the UK, France or other countries in Europe was available. The age distribution of donors in the military was estimated from Department of Defense (DOD) data (2005). The donation rate for military staff was estimated using the blood donor survey data (TSEAC, 2002).

**Variable:** $DR_{R\text{-}(age)}$ - Annual number of recovered plasma donors by age

**Assumption used in the model:** We assumed 3% of recovered plasma donors were grouped by deployment year based on the yearly distribution of military deployment and residence in the UK or Europe during 1980-1996 .(DOD 2005).

$$DR_{R-DOD(age)y} = DR_{R-DOD(age)} \times Perc_{DR-DODy} \quad \text{(IV.C.1.d-4)}$$

**Variable:** $DR_{R(age)}$ - Annual number of recovered plasma donors by age (calculated in section A-IV.B. 2.) grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of recovered plasma donors who have a history of military deployment in the UK and countries in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{R\text{-}DOD(age)}$ - Estimated annual number of recovered plasma donors who have a history of military deployment in the UK and countries in Europe by age.

Assumption used in the model: The same percentage of recovered plasma donors have a history of military deployment as blood donors.

The estimated annual number of recovered plasma donors who have history of military deployment in the UK and countries in Europe by age is represented by the equation:

$$DR_{R\text{-}DOD(age)} = DR_{R(age)} \times Perc_{DR\text{-}DOD} \quad \text{(IV.C.1.d-5)}$$

**Assumption used in the model:** The annual distribution of US military service members and their dependents residing on US military bases in Europe represents the yearly distribution of deployment in the UK and countries in Europe of US military recovered plasma donors

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \quad \text{(IV.C.1.d-6)}$$

## A-IV. C. 1. d. iii. Adjustment of the Relative Risk for the proportional variation in the BSE exposure risk in the UK and the military deployment duration per specific year during the period from 1980 - 1996

**Variable:** $R_{Base}$ - The cumulative vCJD risk of individual military personnel who were deployed and resided in the UK or Europe for the entire period from 1980 through 1996: it is assumed that the accumulated risk is equal to that of a UK resident and is assumed to be 1 (or equal to the UK vCJD prevalence) for this period.

**Assumption used in the model:** The cumulative risk of US military residents in Europe from 1980 through 1996 is 0.35. This estimate is based on the assumption that approximately 35% of the beef consumed by military personnel in Europe between 1980-1996 was imported from the UK (FDA 2002).

**Variable:** $y$ – Year of deployment (same variable used above in A-IV. C. 1. d. ii. a.)

**Variable:** $y_{epi}$ – The specific year of BSE epidemic in the UK.

**Assumption about variable:** The BSE epidemic in the UK was assumed to have started in 1980 and at first was not detected. The first cases of BSE were reported in 1986.

**Variable:** $BSE_{UKy}$ - Number of diagnosed BSE cases in the UK by year from 1980 through 1996

**Data used in the model:** Data are from the World organization for animal health (OIE 2005). Data were not collected for individual years prior to 1997. A total of 446 cases of BSE were reported by European countries (other than the UK) during the time period from 1980 through 1996 and were allocated to individual years by assuming the cases were increasing in a linear fashion by year.

**Variable:** $R_{Basey}$ - Proportional vCJD risk for donors that resided on US military bases in Europe in a specific year

**Assumptions used in the model:**

- The vCJD risk for US military facilities in Europe was present from 1980 through 1996. There was negligible vCJD risk after 1996 – the model assumed the major source of vCJD risk for US military bases in Europe was associated with imported UK beef. When food chain controls were implemented in the UK in 1996 – the model assumed the risk to be negligible.
- It was assumed that the vCJD risk was additive and can be prorated on a yearly base.
- The vCJD risk in a specific year was assumed to be proportional to the reported BSE cases in the UK in that specific year.

Proportional vCJD risk in the US military bases in a specific year was calculated by the equation:

$$R_{Basey} = R_{Base} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.d-7)}$$

**Variable:** $R_{DR\text{-}DODy}$ - Risk of individual military personnel who lived in Europe for a period of two years starting from deployment year y, and assumes the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The model assumed an average of two consecutive years of deployment:

$$R_{DR-DODy} = R_{Basey} + R_{Base(y+1)} \qquad \text{(IV.C.1.d-8)}$$

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (calculated in A-IV. C. 1.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}DOD(age)y}$ - Probability of infection for individual US plasma donor of a specific age group that lived on or near a US military bases in Europe starting from year y with duration of two years

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$Pr_{vCJD-DR-DOD(age)y} = Pr_{vCJD-UK(age)} \times R_{DR-DODy} \qquad \text{(IV.C.1.d-9)}$$

## A-IV. C. 1. d. iv. Number of all US plasma donors potentially infected with vCJD during residence at a US military base in the UK or other countries in Europe from 1980 to 1996

The section estimates the number of Source and recovered plasma donors with a history of deployment in the UK or countries in Europe during the period from 1980 through 1996 and sums the number of vCJD cases that may have vCJD agent in their blood (or be prionemic) at the time of donation from each pathway to derive the total number of all donors potentially infected with vCJD that may have vCJD agent in their blood (or be prionemic).

## A-IV. C. 1. d. iv. a. Potential number of US Source Plasma donors with a history of military deployment in the UK or other countries in Europe from 1980 to 1996 potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD(age)y}$ - Potential number of Source Plasma donors infected during residency on US military facilities in the UK or Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD-S-DOD(age)y} = Binomial(DR_{S-DOD(age)y}, Pr_{vCJD-DR-DOD(age)y}) \qquad \text{(IV.C.1.d-10)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD}$ - Potential number total of Source Plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the expression:

$$DR_{vCJD-S-DOD} = \sum_{y=1980}^{1996} \sum_{age=18-19}^{70-74} DR_{vCJD-S-DOD(age)y} \qquad \text{(IV.C.1.d-11)}$$

## A-IV. C. 1. d. iv. b. Potential number of Source Plasma donors with a history of deployment to a US military facility in the UK or other countries of Europe from 1980 to 1996 in the last half incubation period of the disease

This section estimates the number of Source Plasma donors that may potentially be infected with vCJD who may have vCJD agent in their blood (or be prionemic) at the time of donation.

**Variable:** $y$-The calendar year in which a plasma donor was deployed to a US military base in Europe.

**Assumption used in the model:** This risk assessment assesses the vCJD risk for pdFVIII product made in 2002 but it is assumed that the potential vCJD risk is similar to the present day risk in 2006.

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection/travel and the year 2002 when the plasma was collected.

**Variable:** $Pr\text{-}LH_y$- Probability that the vCJD disease is in the last half of the incubation period of the disease, if infected in year y and the individual has infectious vCJD agent present in their blood and plasma (or was prionemic).

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected is represented by the expression:

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.d-12)}$$

For an individual to be prionemic in 2002, the remaining period of time since infection up to 2002 ($I_{Inf-2002y}$) should be equal to or less than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially or the time between events. In this case it would be the time from infection to the time until the appearance of clinical disease (incubation period of the disease). The distribution is defined by two parameters: one that produces the shape of the curve; and a second generates the scale for the distribution, which in this case is represented by the mean incubation period of 14 years.

**Variable:** $Pr_{LH-y}$ -The probability an individual was prionemic in year 2002-was calculated by using the cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

**Variable:** $DR_{vCJD-S-DODy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment on European bases (calculated in A-IV. C. 1. d. v. a.).

**Variable:** $DR_{vCJD-S-DOD-LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment to US military bases in European countries and in the last half incubation period of the disease.

$$DR_{vCJD-S-DOD-LHy} = Binomial(DR_{vCJD-S-DODy}, Pr_{LH-y}) \qquad \text{(IV.C.1.d-13)}$$

## A-IV. C. 1. d. iv. c. Number of US recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe during the period 1980-1996 and potentially infected with vCJD

**Variable:** $DR_{vCJD-R-DOD(age)y}$ - Potential number of recovered plasma donors infected during deployment and residency on or near US military bases in Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD-R-DOD(age)y} = Binomial(DR_{R-DOD(age)y}, Pr_{vCJD-DR-DOD(age)y}) \qquad \text{(IV.C.1.d-14)}$$

**Variable:** $DR_{vCJD-R-DOD}$ - Potential number of recovered plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the equation:

$$DR_{vCJD-R-DOD} = \sum_{y=1980}^{1996} \sum_{age=12-19}^{70-74} DR_{vCJD-R-DOD(age)y} \qquad \text{(IV.C.1.d-15)}$$

## A-IV. C. 1. d. iv. d. Recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe: Potential number of donors in the last half of vCJD incubation period and vCJD agent is present in the blood

This portion of the model calculates the potential number of vCJD infected recovered plasma donors who are in the last half incubation period of the disease and presumably may contain vCJD agent in their blood (or are prionemic).

**Variable:** $Pr_{LH\text{-}y}$ -The probability a vCJD-infected donor had vCJD agent present in their blood and plasma at the time of donation (was prionemic) in the year 2002 (calculated in A-IV.C.1.d.v.b).

**Variable:** $DR_{vCJD\text{-}R\text{-}DODy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe (calculated in A-IV. C. 1. d. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe and in the last half incubation period of the disease.

$$DR_{vCJD\text{-}R\text{-}DOD\text{-}LHy} = Binomial(DR_{vCJD\text{-}R\text{-}DODy,}\ \mathrm{Pr}_{LH\text{-}y}) \qquad \text{(IV.C.1.d-16)}$$

## A-IV. C. 1. d. iv. e. Number of all vCJD infected plasma donors during deployment to a US military base in a country in Europe from 1980-1996

**Variable:** $\mathbf{DR_{vCJD\text{-}DOD}}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996

$$DR_{vCJD\text{-}DOD} = \sum_{y=1980}^{1996} DR_{vCJD\text{-}S\text{-}DOD} + \sum_{y=1980}^{1996} DR_{vCJD\text{-}R\text{-}DOD} \qquad \text{(IV.C.1.d-17)}$$

**Variable:** $\mathbf{DR_{vCJD\text{-}DOD\text{-}Def}}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

**Assumption used in the model:** Current policy defers individuals who have been deployed or resided on a US military base in Europe from 1980 to 1996 for a cumulative stay of 6 months or more. We assumed all US Department of Defense (DOD) deployments are 6 months or longer, and therefore, all individuals have a history of deployment to a US military base in Europe are deferred.

$$DR_{vCJD\text{-}DOD\text{-}Def} = DR_{vCJD\text{-}DOD} \qquad \text{(IV.C.1.d-18)}$$

## A-IV. C. 1. d. iv. f. Potential number of all plasma donors in the last half of vCJD incubation period

This portion of the model estimates the potential number of all vCJD infected plasma donors with a history of military service posted at a US military base in the UK or countries in Europe fron 1980-1996 who are in the last half incubation period of the disease and their blood and plasma presumably contain infectious vCJD agent (or are prionemic).

**Variable:** $\mathbf{DR_{vCJD\text{-}DOD\text{-}LH}}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and are in the last half incubation period of the disease

$$DR_{vCJD-DOD-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD-LH} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD-LH} \qquad \text{(IV.C.1.d-19)}$$

**Variable:** $\mathbf{DR_{vCJD\text{-}DOD\text{-}Def}}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \qquad \text{(IV.C.1.d-20)}$$

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## A-IV. C. 1. d. i. Percentage of US plasma donors deployed at US military bases in the UK or other countries in Europe during the years 1980 through 1996

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of US blood donors who were military residents in countries in Europe for $\geq$ 6 months from 1980 through 1996.

Assumption used in the model: Approximately 2% of US blood donors have been military residents in European countries between 1980-1996 (TSEAC 2002). There were no data for plasma donors, therefore, data for US blood donors was used to estimate the number of US donors stationed in US military facilities during the period 1980-1996

- The FDA model assumed that the same percentage of plasma donors have been in the military and deployed in European countries as blood donors.

## A-IV. C. 1. d. ii. Number of US plasma donors deployed by Military in the UK or other countries in Europe by year of deployment since 1980

## A-IV. C. 1. d. ii. a. Number of US Source Plasma donors stationed at US military facilities in the UK or countries in Europe during the period from 1980 -1996 by year of deployment

**Variable:** $y$ - Calendar year of deployment

**Variable:** ***DODy*** - Number of US military residents, their family and dependents who resided on US military facilities in Europe by year from 1980 through 1996.

**Assumption used in the model:** The risk of BSE exposure and vCJD infection for donors previously deployed to US military facilities in the UK or countries in Europe after 1996 was assumed to be negligible, because it is assumed that most of the risk was associated with imported UK beef. Food chain controls put in place in the UK after 1996 were assumed to reduce the BSE exposure risk to negligible levels (TSEAC, 2002) and shipment of UK beef to US military facilities had stopped in 1996 or earlier.

**Variable:** ***age*** - age of donors in grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group (same variable used above in section A-IV.C.1.a.ii.)

$$Perc_{DR-DODy} = (DOD_y / \sum_{y=1980}^{1996} DOD_y) \times 100\% \qquad \text{(IV.C.1.d-1)}$$

**Variable:** $DR_{S(age)}$ – Age of donors of Source Plasma (calculated in section A-IV.B. 1.)

**Variable:** $Perc_{DR\text{-}DOD}$ – Percentage of Source Plasma donors who have a history of military deployment in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{S\text{-}DOD(age)}$ - Estimated annual number of Source Plasma donors who have history of military deployment in the UK or Europe by age

**Assumption about variable:** We assumed 3% of Source Plasma donors have a history of military deployment and residence in the UK, France or other countries of Europe during any of the years from 1980-1996 and would have similar donation demographics and characteristics as whole blood donors.

The estimated annual number of Source Plasma donors who have a history of military deployment in Europe by age is represented by the equation:

$$DR_{S-DOD(age)} = DR_{S(age)} \times Perc_{DR-DOD} \qquad \text{(IV.C.1.d-2)}$$

**Variable:** $DR_{S\text{-}DOD(age)y}$ – Number estimated annual number of Source Plasma donors who have resided on military bases in Europe by age and deployment year

$$DR_{S-DOD(age)y} = DR_{S-DOD(age)} \times Perc_{DR-DODy} \qquad \text{(IV.C.1.d-3)}$$

## A-IV. C. 1. d. ii. b. Number of recovered plasma donors with a history of military deployment at US military facilities in the UK or other countries in Europe during the period 1980-1996 by year of deployment

**Variable:** ***age*** - Age of donors grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group. No data for the yearly distribution of deployment of military plasma donors in the UK, France or other countries in Europe was available. The age distribution of donors in the military was estimated from Department of Defense (DOD) data (2005). The donation rate for military staff was estimated using the blood donor survey data (TSEAC, 2002).

**Variable:** $DR_{R\text{-}(age)}$ - Annual number of recovered plasma donors by age

**Assumption used in the model:** We assumed 3% of recovered plasma donors were grouped by deployment year based on the yearly distribution of military deployment and residence in the UK or Europe during 1980-1996 .(DOD 2005).

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \qquad \text{(IV.C.1.d-4)}$$

**Variable:** $DR_{R(age)}$ - Annual number of recovered plasma donors by age (calculated in section A-IV.B. 2.) grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of recovered plasma donors who have a history of military deployment in the UK and countries in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{R\text{-}DOD(age)}$ - Estimated annual number of recovered plasma donors who have a history of military deployment in the UK and countries in Europe by age.

Assumption used in the model: The same percentage of recovered plasma donors have a history of military deployment as blood donors.

The estimated annual number of recovered plasma donors who have history of military deployment in the UK and countries in Europe by age is represented by the equation:

$$DR_{R\text{-}DOD(age)} = DR_{R(age)} \times Perc_{DR\text{-}DOD} \qquad \text{(IV.C.1.d-5)}$$

**Assumption used in the model:** The annual distribution of US military service members and their dependents residing on US military bases in Europe represents the yearly distribution of deployment in the UK and countries in Europe of US military recovered plasma donors

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \qquad \text{(IV.C.1.d-6)}$$

## A-IV. C. 1. d. iii. Adjustment of the Relative Risk for the proportional variation in the BSE exposure risk in the UK and the military deployment duration per specific year during the period from 1980 - 1996

**Variable:** $R_{Base}$ - The cumulative vCJD risk of individual military personnel who were deployed and resided in the UK or Europe for the entire period from 1980 through 1996: it is assumed that the accumulated risk is equal to that of a UK resident and is assumed to be 1 (or equal to the UK vCJD prevalence) for this period.

**Assumption used in the model:** The cumulative risk of US military residents in Europe from 1980 through 1996 is 0.35. This estimate is based on the assumption that approximately 35% of the beef consumed by military personnel in Europe between 1980-1996 was imported from the UK (FDA 2002).

**Variable:** $y$ – Year of deployment (same variable used above in A-IV. C. 1. d. ii. a.)

**Variable:** $y_{epi}$ – The specific year of BSE epidemic in the UK.

**Assumption about variable:** The BSE epidemic in the UK was assumed to have started in 1980 and at first was not detected. The first cases of BSE were reported in 1986.

**Variable:** $BSE_{UKy}$ - Number of diagnosed BSE cases in the UK by year from 1980 through 1996

**Data used in the model:** Data are from the World organization for animal health (OIE 2005). Data were not collected for individual years prior to 1997. A total of 446 cases of BSE were reported by European countries (other than the UK) during the time period from 1980 through 1996 and were allocated to individual years by assuming the cases were increasing in a linear fashion by year.

**Variable:** $R_{Basey}$ - Proportional vCJD risk for donors that resided on US military bases in Europe in a specific year

**Assumptions used in the model:**

- The vCJD risk for US military facilities in Europe was present from 1980 through 1996. There was negligible vCJD risk after 1996 – the model assumed the major source of vCJD risk for US military bases in Europe was associated with imported UK beef. When food chain controls were implemented in the UK in 1996 – the model assumed the risk to be negligible.
- It was assumed that the vCJD risk was additive and can be prorated on a yearly base.
- The vCJD risk in a specific year was assumed to be proportional to the reported BSE cases in the UK in that specific year.

Proportional vCJD risk in the US military bases in a specific year was calculated by the equation:

$$R_{Basey} = R_{Base} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.d-7)}$$

**Variable:** $R_{DR\text{-}DOD_y}$ - Risk of individual military personnel who lived in Europe for a period of two years starting from deployment year y, and assumes the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The model assumed an average of two consecutive years of deployment:

$$R_{DR-DOD_y} = R_{Base_y} + R_{Base(y+1)} \qquad \text{(IV.C.1.d-8)}$$

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (calculated in A-IV. C. 1.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}DOD(age)_y}$ - Probability of infection for individual US plasma donor of a specific age group that lived on or near a US military bases in Europe starting from year y with duration of two years

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$\Pr_{vCJD-DR-DOD(age)_y} = \Pr_{vCJD-UK(age)} \times R_{DR-DOD_y} \qquad \text{(IV.C.1.d-9)}$$

## A-IV. C. 1. d. iv. Number of all US plasma donors potentially infected with vCJD during residence at a US military base in the UK or other countries in Europe from 1980 to 1996

The section estimates the number of Source and recovered plasma donors with a history of deployment in the UK or countries in Europe during the period from 1980 through 1996 and sums the number of vCJD cases that may have vCJD agent in their blood (or be prionemic) at the time of donation from each pathway to derive the total number of all donors potentially infected with vCJD that may have vCJD agent in their blood (or be prionemic).

## A-IV. C. 1. d. iv. a. Potential number of US Source Plasma donors with a history of military deployment in the UK or other countries in Europe from 1980 to 1996 potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD(age)_y}$ - Potential number of Source Plasma donors infected during residency on US military facilities in the UK or Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD-S-DOD(age)_y} = Binomial(DR_{S-DOD(age)_y}, \Pr_{vCJD-DR-DOD(age)_y}) \qquad \text{(IV.C.1.d-10)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD}$ - Potential number total of Source Plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the expression:

$$DR_{vCJD-S-DOD} = \sum_{y=1980}^{1996} \sum_{age=15-19}^{70-74} DR_{vCJD-S-DOD(age)y} \qquad \text{(IV.C.1.d-11)}$$

## A-IV. C. 1. d. iv. b. Potential number of Source Plasma donors with a history of deployment to a US military facility in the UK or other countries of Europe from 1980 to 1996 in the last half incubation period of the disease

This section estimates the number of Source Plasma donors that may potentially be infected with vCJD who may have vCJD agent in their blood (or be prionemic) at the time of donation.

**Variable:** *y*-The calendar year in which a plasma donor was deployed to a US military base in Europe.

**Assumption used in the model:** This risk assessment assesses the vCJD risk for pdFVIII product made in 2002 but it is assumed that the potential vCJD risk is similar to the present day risk in 2006.

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection/travel and the year 2002 when the plasma was collected.

**Variable:** $Pr\text{-}LH_y$- Probability that the vCJD disease is in the last half of the incubation period of the disease, if infected in year y and the individual has infectious vCJD agent present in their blood and plasma (or was prionemic).

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected is represented by the expression:

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.d-12)}$$

For an individual to be prionemic in 2002, the remaining period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or less than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially or the time between events. In this case it would be the time from infection to the time until the appearance of clinical disease (incubation period of the disease). The distribution is defined by two parameters: one that produces the shape of the curve; and a second generates the scale for the distribution, which in this case is represented by the mean incubation period of 14 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual was prionemic in year 2002-was calculated by using the cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

**Variable:** $DR_{vCJD\text{-}S\text{-}DODy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment on European bases (calculated in A-IV. C. 1. d. v. a.).

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment to US military bases in European countries and in the last half incubation period of the disease.

$$DR_{vCJD\text{-}S\text{-}DOD\text{-}LHy} = Binomial(DR_{vCJD\text{-}S\text{-}DODy}, \Pr_{LH-y}) \quad \text{(IV.C.1.d-13)}$$

## A-IV. C. 1. d. iv. c. Number of US recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe during the period 1980-1996 and potentially infected with vCJD

**Variable:** $\mathbf{DR_{vCJD\text{-}R\text{-}DOD(age)y}}$ - Potential number of recovered plasma donors infected during deployment and residency on or near US military bases in Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD\text{-}R\text{-}DOD(age)y} = Binomial(DR_{R\text{-}DOD(age)y}, \Pr_{vCJD\text{-}DR\text{-}DOD(age)y}) \quad \text{(IV.C.1.d-14)}$$

**Variable:** $\mathbf{DR_{vCJD\text{-}R\text{-}DOD}}$ - Potential number of recovered plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the equation:

$$DR_{vCJD\text{-}R\text{-}DOD} = \sum_{y=1980}^{1996} \sum_{age=18\text{-}19}^{70\text{-}74} DR_{vCJD\text{-}R\text{-}DOD(age)y} \quad \text{(IV.C.1.d-15)}$$

## A-IV. C. 1. d. iv. d. Recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe: Potential number of donors in the last half of vCJD incubation period and vCJD agent is present in the blood

This portion of the model calculates the potential number of vCJD infected recovered plasma donors who are in the last half incubation period of the disease and presumably may contain vCJD agent in their blood (or are prionemic).

**Variable:** $Pr_{LH\text{-}y}$ -The probability a vCJD-infected donor had vCJD agent present in their blood and plasma at the time of donation (was prionemic) in the year 2002 (calculated in A-IV.C.1.d.v.b).

**Variable:** $DR_{vCJD\text{-}R\text{-}DODy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe (calculated in A-IV. C. 1. d. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe and in the last half incubation period of the disease.

$$DR_{vCJD-R-DOD-LHy} = Binomial(DR_{vCJD-R-DODy,} \Pr_{LH-y}) \quad \text{(IV.C.1.d-16)}$$

## A-IV. C. 1. d. iv. e. Number of all vCJD infected plasma donors during deployment to a US military base in a country in Europe from 1980-1996

**Variable: $DR_{vCJD-DOD}$** - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996

$$DR_{vCJD-DOD} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD} \quad \text{(IV.C.1.d-17)}$$

**Variable: $DR_{vCJD-DOD-Def}$** - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

**Assumption used in the model:** Current policy defers individuals who have been deployed or resided on a US military base in Europe from 1980 to 1996 for a cumulative stay of 6 months or more. We assumed all US Department of Defense (DOD) deployments are 6 months or longer, and therefore, all individuals have a history of deployment to a US military base in Europe are deferred.

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \quad \text{(IV.C.1.d-18)}$$

## A-IV. C. 1. d. iv. f. Potential number of all plasma donors in the last half of vCJD incubation period and have vCJD agent present in the blood

This portion of the model estimates the potential number of all vCJD infected plasma donors with a history of military service posted at a US military base in the UK or countries in Europe fron 1980-1996 who are in the last half incubation period of the disease and their blood and plasma presumably contain infectious vCJD agent (or are prionemic).

**Variable: $DR_{vCJD-DOD-LH}$** - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and are in the last half incubation period of the disease

$$DR_{vCJD-DOD-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD-LH} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD-LH} \quad \text{(IV.C.1.d-19)}$$

**Variable: $DR_{vCJD-DOD-Def}$** - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \quad \text{(IV.C.1.d-20)}$$

**Variable:** $DR_{Tot}$ - (calculated in section A-IV.B.3.) Annual number of plasma donors

**Variable:** $DR_S$ - (calculated in section A-IV.B.1.) Annual number of Source Plasma donors

**Variable:** $DR_R$ - (calculated in section A-IV.B.2.) Annual number of recovered plasma donors

**Variable:** $Perc_{DR\text{-}Eurob}$ - Percentage of blood donors who were Euroblood recipients

**Assumption used in the model:** 1.2% plasma donors were Euroblood recipients

**Variable:** $DR_{Eurob}$ - Annual number of plasma donors who were Euroblood recipients

$$DR_{Eurob} = DR_{Tot} \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-1)}$$

**Variable:** $DR_{S\text{-}Eurob}$ - Annual number of Source Plasma donors who were Euroblood recipients

**Assumption used in the model:** We assumed 1.2% of US Source Plasma donors were Euroblood recipients

$$DR_{S-Eurob} = DR_S \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-2)}$$

**Variable:** $DR_{R\text{-}Eurob}$ - Annual number of recovered plasma donors who were Euroblood recipients – represented by the equation:

$$DR_{R-Eurob} = DR_R \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-3)}$$

## A-IV. C. 1. e. Annual number of US plasma donors who have been Euroblood recipients

## A-IV. C. 1. e. i. Annual number of US plasma donors who have been Euroblood recipients

## A-IV. C. 1. e. ii. Annual number of potential vCJD-infected Euroblood donors and estimated annual units of Euroblood potentially containing vCJD agent

This section of the model estimates the quantity of Euroblood units predicted to have been transfused into US plasma donors in a one year period, the number of European donors involved, the number of possible vCJD infected European Euroblood donors, and the total quantity of vCJD infected units given by the Euroblood donors.

**Variable:** $DNBl_{UK(age)}$ - Blood donations in the UK by age of donors.

**Data used in the model:** Information about UK blood donors was provided by CDSC (2005).

**Variable:** $Perc_{DN\text{-}UK(age)}$ - Percentage distribution of the blood donations in the UK by donor age, and is represented by equation:

$$Perc_{DN-UK(age)} = DN_{BI-UK(age)} / \sum_{age=18}^{>65} DN_{BI-UK(age)} \quad \text{(IV.C.1.e-5)}$$

**Variable:** $EUBL_{(age)}$ - Units of Euroblood that were donated by a specific age group of European donors and transfused into US plasma donors

Total units of Euroblood received by US plasma donors of one year period is allocated by age of European donors based on the age distribution of UK blood donors

Assumption used in the model: We assumed the age distribution for Euroblood donors is the same as UK blood donors

$$EUBL_{(age)} = EUBL_{Tot} \times Perc_{DN-UK(age)} \quad \text{(IV.C.1.e-6)}$$

**Variable:** $DR_{EUBL(age)}$ – The number of European donors were grouped by age in five-year increments (e.g., 20-24 yrs, etc) and the 18-19 yr old group that have contributed Euroblood that may have been transfused into US plasma donors per one year period

Each European donor may give multiple donations in a single year; however the chance of more than one donation from same donor being shipped to the US and used by US plasma donors is expected to be small.

Assumption used in the model: Each unit of Euroblood received by US plasma donors of one year-period came from different European donors and is expressed by the equation:

$$DR_{EUBL(age)} = EUBL_{(age)} \quad \text{(IV.C.1.e-7)}$$

The probability of infection of individual European blood donors is calculated below based on the probability of infection per individual UK resident and the relative risk of a resident in a country elsewhere in Europe (other than France) compared to the risk of a UK resident.

**Variable:** $R_{EU}$ – The cumulative risk of an individual European resident from 1980 till the present; assumes that the cumulative risk of a UK individual from 1980 through 1996 is 1 (same variable as used in (A-IV.C.1.c.iii)

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (same variable as used in (A-IV.C.1. c. iv.).

**Variable:** $Pr_{vCJD\text{-}EU(age)}$-Probability of infection for an individual European resident of a specific age group.

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure:

$$\Pr_{vCJD\ -EU\ (age\ )} = \Pr_{vCJD\ -UK\ (age\ )} \times R_{EU} \qquad \text{(IV.C.1.e-7)}$$

**Variable:** $DR_{vCJD\text{-}EUBL(age)}$-Annual number of infected European donors who contributed Euroblood that was transfused into US plasma donors during a one-year period by age group.

Number of infected Euroblood donors among each age group was estimated using a binomial distribution function with the estimated total number of donors in the subgroup ($DR_{EUBL(age)}$ estimated in section A-IV.C. 1.e. ii) and the probability of infection for the individual of this age group of Euroblood donors ($Pr_{vCJD\text{-}DR\text{-}EUBL(age)}$ estimated in this section A-IV. C. 1. e. ii) as parameters of the distribution.

$$DR_{vCJD-EUBL(age)} = Bionomial(DR_{EULL(age)}, \Pr_{vCJD-EU(age)}) \qquad \text{(IV.C.1.e-8)}$$

**Variable:** $EUBL_{vCJD}$ - Total units of infected Euroblood received by US plasma donors of one year period

Potential infected European donor may give multiple donations in a single year, however the chance of more than one donation being from a single infected European donor being shipped to the US, and used by US plasma donors is expected to be small.

Assumption about variable: One infected European donor produces one unit of infected Euroblood.

$$EUBL_{vCJD} = \sum_{age=18-19}^{>65} DR_{vCJD-EUBL(age)} \qquad \text{(IV.C.1.e-9)}$$

## A-IV. C. 1. e. iii. Annual number of plasma donors potentially infected with vCJD via transfusion with Euroblood

## A-IV. C. 1. e. iii. a. Annual potential number of vCJD infected plasma donors

**Variable:** $Pr_{vCJD\text{-}EUBL}$ - Probability a single unit of Euroblood contains vCJD agent

$$\Pr_{vCJD-EUBL} = EUBL_{VCJD} / EUBL_{Tot} \qquad \text{(IV.C.1.e-10)}$$

**Variable:** $Pr_{vCJD\text{-}EUBL\text{-}Recip}$ - Probability a Euroblood recipient is infected with vCJD

**Assumption used in the model:** We assumed that a Euroblood recipient is likely infected if he/she receives one unit or more of blood blood from a vCJD-infected donor.

$$\Pr_{vCJD-EUBL-recip} = 1 - Binomdist(0, EUBL_{avg}, \Pr_{vCJD-EUBL}, false) \qquad \text{(IV.C.1.e-11)}$$

The Excel function Binomdist (n, N, p, false) calculates the probability of n "success" outcomes in a test, if the outcome of each trial of the test is either "success" or "failure", the probability of getting the outcome of "success" in an individual trial throughout the test is a constant $p$, and the number of trials in the test is $N$. In the problem we addressed here the outcomes are the probability of a donation being from a Euroblood recipient that receives a donation that is either infected or not infected. In equation IV.C.1.e-11, $Binomdist(0, EUBL_{avg}, Pr_{vCJD\text{-}EUBL}, false)$ calculated the probability of a recipient receiving no infected unit ($n$=0), under the condition that average number of units received by a recipient is $EUBL_{avg}$ ($N$=$EUBL_{avg}$) and probability a single unit of Euroblood being infected is $Pr_{vCJD\text{-}EUBL}$ ($p$=$Pr_{vCJD\text{-}EUBL}$).

The number of Source and recovered plasma donors infected through transfusion with Euroblood was estimated using a binomial distribution function with the estimated total number of source and recovered plasma donors who have received Euroblood ($DR_{S\text{-}EUBL}$ and $DR_{R\text{-}EUBL}$ estimated in section A-IV.C. 1.e. ii. ) and the probability of infection for the individual Euroblood recipient ($Pr_{vCJD\text{-}EUBL\text{-}receip}$ estimated in section A-IV. C. 1. e. iii) as parameters of the distribution. The total estimated number of potential plasma donors infected due to transfusion using Euroblood is the sum of potential infected source and recovered plasma donors.

**Variable:** $DR_{vCJD\text{-}S\text{-}EUBL}$ - Annual Number of Source Plasma donors being infected due to transfusion with a Euroblood unit:

$$DR_{vCJD-S-EUBL} = Binomial(DR_{S-EUBL}, \Pr_{vCJD-EUBL-recip}) \quad \text{(IV.C.1.e-12)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EUBL}$ - Annual number of recovered plasma donors possibly infected via transfusion with a unit of Euroblood

$$DR_{vCJD-R-EUBL} = Binomial(DR_{R-EUBL}, \Pr_{vCJD-EUBL-recip}) \quad \text{(IV.C.1.e-13)}$$

**Variable:** $DR_{vCJD\text{-}EUBL}$ - Annual number of all plasma donors possibly infected through transfusion with a unit of Euroblood

$$DR_{vCJD-EUBL} = DR_{vCJD-S-EUBL} + DR_{vCJD-R-EUBL} \quad \text{(IV.C.1.e-14)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Def}$- Annual number of plasma donors possibly infected through transfusion with a unit of Euroblood and meet deferral criteria and presumably deferred from donation.

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Res}$- Annual number of plasma donors potentially infected via transfusion with a unit of Euroblood and does not meet deferral criteria and likely not deferred from donation.

Under current blood donation policies recipients of Euroblood are not deferred and represented by the expressions:

$$DR_{vCJD-EUBL-Def} = 0 \quad \text{(IV.C.1.e-15)}$$

$$DR_{vCJD-EUBL-Res} = DR_{vCJD-EUBL} \quad \text{(IV.C.1.e-16)}$$

## A-IV. C. 1. e. iii. b. Annual number of plasma donors that received Euroblood and are potentially infected and vCJD agent is present in the blood

**Assumption used in the model:** All infected Euroblood recipients have vCJD agent present in their blood (prionemic)

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Pn}$ - Annual number of plasma donors infected via transfusion using Euroblood and are prionemic in 2002

$$DR_{vCJD-EUBL-Pn} = DR_{vCJD-EUBL} \quad \text{(IV.C.1.e-17)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Def\text{-}Pn}$- Annual number of plasma donors infected via transfusion using Euroblood, and may have vCJD agent present in their blood and plasma (prionemic) in 2002 and meet deferral criteria

$$DR_{vCJD-EUBL-Def-Pn} = DR_{vCJD-EUBL-Def} \quad \text{(IV.C.1.e-18)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Res\text{-}Pn}$- Annual number of plasma donors who are possibly infected with vCJD via transfusion with Euroblood, that had vCJD agent present in their blood and plasma (prionemic) in 2002 and did not meet deferral criteria (likely not deferred)

$$DR_{vCJD-EUBL-Res-Pn} = DR_{vCJD-EUBL-Res} \quad \text{(IV.C.1.e-19)}$$

## A-IV. C. 1. f. Total number all plasma donors who may potentially be infected with vCJD through all sources of exposure and vCJD agent is present in the blood

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn}$ - Estimated total annual number of Source Plasma donors infected with vCJD who potentially had the agent present in blood and plasma (prionemic) in 2002:

$$DR_{vCJD-S-Pn} = DR_{vCJD-S-UK-LH} + DR_{vCJD-S-FR-HL} + DR_{vCJD-S-DOD-LH} + DR_{vCJD-S-EUBL-Pn} \quad \text{(IV.C.1.f-1)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}Pn}$ - Estimated total annual number of recovered plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-R-Pn} = DR_{vCJD-R-UK-LH} + DR_{vCJD-R-FR-HL} + DR_{vCJD-R-EU-LH} + DR_{vCJD-R-DOD-LH} + DR_{vCJD-R-EUBL-Pn}$$

(IV.C.1.f-2)

**Variable:** $DR_{vCJD\text{-}Pn}$ - Total annual number of all US plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-Pn} = DR_{vCJD-S-Pn} + DR_{vCJD-R-Pn} \quad \text{(IV.C.1.f-3)}$$

## A-IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in the blood and who may not be deferred by questionnaire screening

## A-IV. C. 2. a Annual number of US Source Plasma donors potentially infected and vCJD agent is present in the blood that may not be deferred by questionnaire screening

**Variable:** $Eff_{Def}$ - Effectiveness of US donor deferral policy

**Assumption about variable**: Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Variable:** $DR_{vCJD\text{-}S\text{-}Def\text{-}Pn}$ - Estimated annual number of Source Plasma donors potentially infected with and having vCJD agent present in blood and plasma (prionemic) that are deferred by current policy

$$DR_{vCJD\text{-}S\text{-}Def\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-1)}$$

**Assumption about variable**: This population includes the potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have long-term travel history to the UK (≥3 mo), and France (≥ 5 yrs); and a history of military deployment (or military dependent, etc.) in Europe from 1980 – 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}Res}$ - Estimated annual number of Source Plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that have short term travel history (UK (<3 mo), and France and/or Europe (< 5 yrs); and not deferred by deferral policy.

$$DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-2)}$$

**Assumption about variable**: This population includes the potential Source Plasma donors with vCJD with agent present in blood and plasma (prionemic) that have short-term travel history to the UK (< 3 mo), France (< 5 yrs), and a history of receiving Euroblood.

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn\text{-}NR}$ - Annual total number of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) and were not removed by deferral screening

**Assumption used in model:** This includes potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) who meet deferral criteria but for a variety of reasons are not deferred.

$$DR_{vCJD\text{-}S\text{-}Pn\text{-}NR} = DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}Def\text{-}Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-3)}$$

## A-IV. C. 2. b Annual number of US recovered plasma donors potentially infected and vCJD agent is present in the plasma that may not be deferred by questionnaire screening

**Variable:** $Eff_{Def}$ - Effectiveness of donor deferral policy

**Assumption about variable:** Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Variable:** $DR_{vCJD\text{-}R\text{-}Def\text{-}Pn}$ - Estimated annual number of potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) who are deferred by current policy

$$DR_{vCJD\text{-}R\text{-}Def\text{-}Pn} = DR_{vCJD\text{-}R\text{-}UK\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}EU\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-4)}$$

**Assumption about variable:** Model includes potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) that have long term travel history to the UK (≥3 mo), France (≥ 5 yrs), and Europe (≥5 yrs); and history of military deployment, military dependent or related travel or residence in Europe.

There is a possibility that some individuals that traveled to the UK, France, and other countries in Europe since 1980 stayed for periods of time that were shorter than the deferral period, were exposed to BSE agent, and were infected with vCJD. These individuals represent a source of residual risk – or the remaining risk after interventions (in this case donor deferral policies) are applied. The section below addresses the calculation of residual risk for non-deferred at risk donors that traveled for periods of time that were shorter than recommended guidelines.

**Variable:** $DR_{vCJD\text{-}R\text{-}Res}$ - Annual number of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have short term travel history and are not covered by and deferred by deferral policy.

$$DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-2)}$$

**Assumption about variable:** Estimation of the possible vCJD residual risk includes potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have short-term travel history to the UK (< 3 mo), France (< 5 yrs), history of travel to Europe and history of receiving Euroblood.

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn\text{-}NR}$ - Annual total of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) and were not removed by deferral screening

**Assumption used in model:** This includes all potential donors with vCJD agent present in blood and plasma (prionemic) that do not meet deferral criteria and who meet deferral criteria but wrongly not deferred.

$$DR_{vCJD-S-Pn-NR} = DR_{vCJD-S-Res-Pn} + DR_{vCJD-S-Def-Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-3)}$$

**Variable: $DR_{vCJD-R-Pn-NR}$** - Annual total of potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) not removed by deferral screening

$$DR_{vCJD-R-Pn-NR} = DR_{vCJD-R-Res-Pn} + DR_{vCJD-R-Def-Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-4)}$$

## A-IV. C. 2. c Total number of all US plasma donors potentially infected and vCJD agent is present in the plasma but may not be deferred by questionnaire screening

The total number of all US plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that may not be deferred by questionnaire screening was determined by summing the estimates generated for both Source and recovered plasma donors that may not be deferred by current screening procedures, and is described by the equation:

$$DR_{vCJD-Pn-NR} = DR_{vCJD-S-Pn-NR} + DR_{vCJD-R-Pn-NR} \quad \text{(IV.C.2-5)}$$

## A-IV. C. 3. Total number of all US plasma donations potentially containing vCJD agent

**Variable: $Freq_{DN-S}$**–Average frequency of donations from a single Source Plasma donor who contributes Source Plasma for FVIII manufacture (times/year)

**Variable: $DN_{vCJD-S}$** - Annual number of potential vCJD donations of Source Plasma

$$DN_{vCJD-S} = DR_{vCJD-S-Pn-NR} \times Freq_{DN-S} \quad \text{(IV.C.2-6)}$$

Assumption used in the model: The average frequency of donations from a single Source Plasma donor who contributes Source Plasma for a FVIII plasma pool used in manufacturing ranges from ----- and most likely to be --- based on data from pdFVIII manufacturers. [FOI REVIEW]

**Variable: $Freq_{DN-R}$**–The average frequency of donations from a single recovered plasma donor who contribute plasma for pdFVIII manufacture (times/year) is 1.

**Variable: $DN_{vCJD-R}$** - Annual number of potential vCJD donations of recovered plasma

$$DN_{vCJD-R} = DR_{vCJD-R-Pn-NR} \times Freq_{DN-R} \quad \text{(IV.C.2-7)}$$

Assumption used in the model: The average frequency of donations from a single blood donor that contributes recovered plasma for pdFVIII manufacture is 1.

## A-IV. C. 4. Probability that a US plasma donation potentially contained vCJD agent among all donations

**Variable: $Pr(DN_{vCJD-S})$** - Probability a donation of Source Plasma contained vCJD agent

**Variable: $DN_s$**-Annual units of Source Plasma used to make pdFVIII (calculated in A-IV. B. 1)

$$Pr(DN_{vCJD-S}) = DN_{vCJD-S} / DN_S \qquad \text{(IV.C.4-1)}$$

**Variable: $Pr(DN_{vCJD-R})$** - Probability a donation of recovered plasma contained vCJD agent.

**Variable: $DN_R$**-Annual number of units of recovered plasma used to make pdFVIII from plasma collected in the US (calculated in A-IV. B. 2).

$$Pr(DN_{vCJD-R}) = DN_{vCJD-R} / DN_R \qquad \text{(IV.C.4-2)}$$

## A-IV. C. 5. Probability of a Source or recovered plasma pool potentially containing a vCJD donation(s)

### A-IV. C. 5. a. *Probability that a plasma pool may contain a specific number of* vCJD donations

Assumption used in the model: Consistent with manufacturing practices in which commingling of Source and recovered plasma is uncommon, the risk assessment considered plasma pools to consist entirely of only Source Plasma donations or only recovered plasma donations.

**Variable: $n_{vCJD-DN-pool}$** - Designated number of vCJD donors in a single plasma pool.

Assumption used in the model: The number of vCJD donations in a single vCJD pool could be 0, 1, 2, 3 or 4, but because of the low prevalence of vCJD most of the time there would be 0 vCJD donations in a pool

**Variable: $DR_{pool-S}$** -Size of Source Plasma pool (donors/pool)

**Data used in the model:** Based on information provided to the FDA by pdFVIII manufacturers, an individual Source Plasma pool may contain 6,000 to 60,000 donors [FOI REVIEW]. A statistical distribution representing the variation in the size (number of donations per pool) of plasma pools used in the manufacture of pdFVIII was generated by combining information on pool size with information on the percentage of market share for several individual pdFVIII products.

**Variable: $Pr(n_{vCJD-DR-pool-S})$**- Probability a Source Plasma pool containing $n_{vCJD-DN-pool}$ infected donations ($n_{vCJD-DR-pool}$=0, 1, 2, 4)-are determined by density frequency of $DR_{pool-S}$ at $X=n_{vCJD-DR-pool}$

**Variable:** $DN_{pool\text{-}R}$ -Size of recovered plasma pool (donors/pool)

**Data used in the model:** Based on information from manufacturers, individual Source Plasma pool may contain 6,000 to 60,000 donors. [FOI REVIEW].

**Variable:** $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$- Probability a Source Plasma pool containing $n_{vCJD\text{-}DN\text{-}pool}$ infected donations ($n_{vCJD\text{-}DR\text{-}pool}$ =0, 1, 2, 4)-are determined by density frequency of $DR_{pool\text{-}S}$ at $X=n_{vCJD\text{-}DR\text{-}pool}$

**Variable:** $DN_{pool\text{-}R}$ -Size of recovered plasma pool (donors/pool)

**Data used in the model:** Based on information provided to the FDA by pdFVIII manufacturers, an individual recovered plasma pool may contain 150,000 to 360,000 donors [FOI REVIEW]. As with Source Plasma pools described above, a statistical distribution representing the variation in the size (number of donations per pool) of plasma pools used in the manufacture of pdFVIII was generated.

**Assumptions used in the model:** The size of recovered plasma pools was represented in the model by using a uniform distribution ranging from 150,000 to 360,000 donations per pool (Figure 4-3) [FOI REVIEW]. – representing the range of pool sizes used by manufacturers of pdFVIII. The uniform distribution provided the best fit for the range of possible recovered plasma pool sizes that may be used in the US to manufacture pdFVIII.

**Variable:** $Pr(n_{vCJD\text{-}DN\text{-}pool\text{-}R})$-Probability a recovered plasma pool containing $n_{vCJD\text{-}DN\text{-}pool}$ infected donations ($n_{vCJD\text{-}DN\text{-}pool}$ =0, 1, 2, 4)-are determined by density frequency of $DR_{pool\text{-}R}$ at $X=n_{vCJD\text{-}DR\text{-}pool}$

## A-IV. C. 5. b. Probability a plasma pool may potentially contain a vCJD donation(s)

**Variable:** $Pr(vCJD\text{-}pool_S)$-Probability of a Source Plasma pool containing one or more vCJD donations

$$\Pr(vCJD\_pool_S) = 1 - \Pr(n_{vCJD-DN-poolS} = 0) \quad \text{(IV.C.5-1)}$$

**Variable:** $Pr(vCJD\text{-}pool_R)$-Probability of a recovered plasma pool containing one or more vCJD donations

$$\Pr(vCJD\_pool_R) = 1 - \Pr(n_{vCJD-DN-pool\,R} = 0) \quad \text{(IV.C.5-2)}$$

**Variable:** $Pr(vCJD\text{-}pool)$-The Probability that a plasma pool (including Source and recovered plasma pools) contained one or more vCJD donations- The distribution for pool size (or number of donations per pool) incorporated information on pool size.

**Variable:** $Perc_S$ – Percentage of Source Plasma pools used to manufacture pdFVIII in the US

**Variable:** $Perc_R$ – Percentage of recovered plasma pools used to manufacture pdFVIII in the US

**Assumption used in the model:** Estimates suggest that approximately --- of pdFVIII products were made from Source Plasma, and --- were made from recovered plasma.

The probability of either a vCJD donation being present in a Source Plasma pool is represented by the variable ***Pr(vCJD-pool$_S$*** and the probability of a vCJD donation being present in a recovered plasma pool is represented by the variable, ***Pr(vCJD-pool$_R$***, which was calculated in the section above. A discrete distribution (X1, X2; p1, p2) represents two discrete values for the probabilities that a pool may contain a vCJD donation, X1(or ***Pr(vCJD-pool$_S$***) and X2 (or ***Pr(vCJD-pool$_R$***) and the associated probabilities of each value occurring with the probabilities, p1 and p2, respectively. Based on the assumptions above that Source Plasma pools are used more frequently in the manufacture of pdFVIII and, on average contain fewer donations, the probability of a Source Plasma pool containing vCJD agent is different from the probability a recovered plasma pool containing vCJD agent. Overall probability of a single plasma pool (including source and recovered plasma pool) containing vCJD agent is a probability weight based on the percentages of the two types of plasma pools (--- for Source and --- for recovered plasma pools) used to make pdFVIII. *Pr(vCJD-pool)* is sampled from ***Pr(vCJD-pool$_S$*** and ***Pr(vCJD-pool$_R$*** using the discrete distribution:

$$\Pr(vCJD - pools) = Discrete(\ Pr(vCJD\text{-}pool\text{-}s),\ Pr(vCJD\text{-}pool\text{-}R);\ Perc_S, Perc_R)\ ) \qquad \text{(IV.C.5-3)}$$

**or**

$$\Pr(vCJD - pools) = Discrete(\ Pr(vCJD\text{-}pool\text{-}s),\ Pr(vCJD\text{-}pool\text{-}R);\ ---,\ ---)\ )$$

## A-IV. D. Annual total number of all plasma pools and number of plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US

### A-IV. D.1. Annual amount of pdFVIII distributed in the US

**Variable:** $IU_{FVIII}$ -Annual number of all units of human pdFVIII manufactured and distributed in the US .

**Data used in the model**: Based on data provided to FDA from manufacturers, a total of --- million units of pdFVIII was made and distributed in the US. [FOI REVIEW].

**Variable:** $Perc_S$ – Represents the percentage of pdFVIII assumed in the model to be made from Source Plasma (same as variable used in A-IV. C. 5. b.)

**Variable:** $Perc_R$ – Represents the percentage of pdFVIII assumed in the model to be made from recovered plasma (same as variable used in A-IV. C. 5. b.)

**Variable:**$IU_{FVIII\text{-}S}$ –The total annual number of units of pdFVIII made from Source Plasma and is represented by the equation:

$$IU_{FVIII-S} = IU_{FVIII} \times Perc_s \qquad \text{(IV.D.1-1)}$$

**Variable:**$IU_{FVIII\text{-}R}$ -The total annual number of units of pdFVIII made from recovered plasma and is represented by the equation:

$$IU_{FVIII-R} = IU_{FVIII} \times Perc_R \qquad \text{(IV.D.1-2)}$$

## A-IV. D. 2. Annual total number of all plasma pools used to make pdFVIII and plasma pools with vCJD agent

The total number of plasma pools used to make pdFVIII in the US each year can be back-calculated from the total number of units of human plasma-derived pdFVIII distributed in the US each year. Based on information described in earlier sections, it was assumed that approximately — of the total pdFVIII supply distributed annually in the US is manufactured from Source Plasma and — from recovered plasma pools. Information on pool size (number of donors), average number of donations per donor, size of individual recovered plasma donations (200 mls) and Source Plasma donations (700 mls) were used to first determine the amount of plasma present in a pool. Then, data on the average yield of pdFVIII per liter of plasma (187 IU), was used to calculate the total number of Source and recovered plasma pools and the results were summed to determine the total number of plasma pools used to manufacture pdFVIII in the US each year. The total number (or percentage) of plasma pools potentially containing vCJD agent was determined in the model based on pool size and the probability that a pool contained a vCJD agent.

### A-IV. D. 2. a. Amount plasma per pool

**Variable:** $\mathbf{DN_{V\text{-}s}}$ – Volume of single unit Source Plasma (ml).

**Variable:** $\mathbf{DR_{pool\text{-}s}}$ – Number donors per Source Plasma pool (same variable as used in A-IV.C. 5).

**Variable:** $\mathbf{Freq_{DN\text{-}S}}$ –Average frequency of donations from a single Source Plasma donor that contribute Source Plasma for pdFVIII manufacture (same variable as used in A-IV.C. 3).

**Variable:** $\mathbf{V_{pool\text{-}S}}$ — Volume of a Source Plasma pool (ml).

$$V_{pool-S} = DR_{pool-S} \times Freq_{DN-S} \times DN_{V-S} \quad \text{(IV. D. 2-1)}$$

**Variable:** $\mathbf{DN_{V\text{-}R}}$ – Volume of single unit recovered plasma (ml).

**Variable:** $\mathbf{DR_{pool\text{-}R}}$ – Number donors per recovered plasma pool (same variable as used in A-IV.C. 5. a.)

**Variable:** $\mathbf{Freq_{DN\text{-}S}}$ –Average frequency of donations from a single recovered plasma donor that contribute recovered plasma for pdFVIII manufacture (same variable as used in A-IV.C. 3)

**Variable:** $\mathbf{V_{pool\text{-}S}}$ — Volume of a recovered plasma pool (ml)

$$V_{pool-R} = DR_{pool-R} \times Freq_{DN-R} \times DN_{V-R} \text{ (IV. D. 2-2)}$$

## A-IV. D. 2. b. Annual number of plasma pools used to manufacture pdFVIII in the United States

**Variable:**$IU_{FVIII-S}$ -Annual units of pdFVIII made from Source Plasma (calculated in A-IV. D. 1)

**Variable:** $Y_{avg}$–Average yield of pdFVIII (IU/L plasma)

**Assumption used in the model:** Based on the data provided by WFH (1998) and FDA-CBER (2003) we assumed average yield of pdFVIII (including high purity and intermediated purity pdFVIII) being 187 IU per liter plasma.

The total number of Source Plasma pools and recovered plasma pools used each year in manufacturing US pdFVIII are calculated separately in the model. Estimates from each type of pool are then summed to get a total value for all pools.

**Variable: $Pool_s$**–Annual number Source Plasma pool used to make pdFVIII

$$Pool_s = Round((IU_s / Y_{avg}) / (V_{pool-s} / 1000)) \qquad \text{(IV. D. 2-3)}$$

**Variable:**$IU_{FVIII-R}$ -Annual units of pdFVIII made from recovered plasma (calculated in A-IV. D. 1.).

**Variable: $Pool_R$**–Annual number of recovered plasma pools used to make pdFVIII

$$Pool_R = Round((IU_R / Y_{avg}) / (V_{pool-R} / 1000)) \qquad \text{(IV. D. 2-4)}$$

Finally, the number of possible Source and recovered plasma pools are summed to generate the total number of plasma pools used in the manufacture of pdFVIII in the US.

**Variable: Pool**–Annual total number of plasma pool used to make pdFVIII

$$Pool = Pool_S + Pool_R \qquad \text{(IV. D. 2-5)}$$

## A-IV. D. 2. c. Annual number vCJD plasma pools used to manufacture pdFVIII in the United States

Annual number of vCJD pools is expected to be low because the US vCJD prevalence, even among donors that traveled to the UK, France or other countries in Europe since 1980, is likely very low and presumably varies from year to year. A binomial distribution (n, p) is used to reflect the variation in the number of vCJD pools present in a single year. A binomial distribution is usually used when the number of positive observations (p) or in this case the number of vCJD containing pools is very low compared to the total number of pools (n).

**Variable:** ***Pr(vCJD-pool$_s$)***- Probability of a Source Plasma pool containing vCJD agent

**Variable: $Pool_{vCJD-S}$** – Annual number Source Plasma pools that contain vCJD agent used to make pdFVIII

$$Pool_{vCJD-S} = Binomial(Pool_{S,} \Pr(vCJD - pool_S) \quad \text{(IV. D. 2-6)}$$

**Variable: *Pr(vCJD-pool$_R$)*-** Probability of a recovered plasma pool containing vCJD agent

**Variable: Pool$_{vCJD-R}$** – Annual number of recovered plasma pools that contain vCJD agent used to make pdFVIII

$$Pool_{vCJD-R} = Binomial(Pool_{R,} \Pr(vCJD - pool_R) \quad \text{(IV. D. 2-7)}$$

**Variable: Pool$_{vCJD}$** – Annual total plasma pools that contains vCJD agent used to make pdFVIII

$$Pool_{vCJD} = Pool_{vCJD-S} + Pool_{vCJD-R} \quad \text{(IV. D. 2-8)}$$

## A-IV. D. 3. Percentage of pools potentially containing vCJD agent

**Variable: Perc $_{vCJD-S-pool}$** -Percentage Source Plasma pools used to make pdFVIII that contains vCJD donations

$$Perc_{vCJD-S-pool} = (Pool_{vCJD-S} / Pool_S) \times 100\% \quad \text{(IV. D. 3-1)}$$

**Variable: Perc $_{vCJD-R-pool}$** -Percentage recovered plasma pools used to make pdFVIII that contains vCJD donations

$$Perc_{vCJD-R-pool} = (Pool_{vCJD-R} / Pool_R) \times 100\% \quad \text{(IV. D. 3-2)}$$

**Variable: Perc $_{vCJD-pool}$**–Overall percentage plasma pools used to make pdFVIII that contains vCJD donations

## A-IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD

## A-IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD

**Variable: $I_{bl}$** – Represents the i.c. $ID_{50}$ present in the blood of individual infected donor ($ID_{50}$/ml) in the last half of the incubation period of vCJD.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5th percentile of 2 i.c. $ID_{50}$ per ml, a median of 12 i.c. $ID_{50}$ per ml, a 95th percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected (Wadsworth, 2001).
**Variable:** $I_{pl\text{-}perc}$ – Percent (%) i.v. $ID_{50}$s associated with plasma

Studies in animal models have shown that greater than 50% of transmissible spongiform encephalopathy agent present in whole blood is associated with plasma. Experiments by Gregori *et al.* (2004) using a hamster – sheep scrapie model showed that approximately 58% of infectivity in whole blood is associated with plasma.

**Assumption used in the model:** The model assumes that 58% of infectivity is associated with plasma.

**Assumption used in the model:** Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

**Variable:** $DN_V$-Volume of one unit of plasma, depending on plasma type (same as $DN_{V\text{-}S}$ used in A-IV. D. 2 for Source Plasma, same as $DN_{V\text{-}R}$ used in A-IV. D. 2. for recovered plasma)

**Variable:** $I_{DN}$-Quantity of vCJD agent in one donation of infected plasma (i.v. $ID_{50}$/ml)

$$I_{DN} = I_{bl} \times DN_V \times I_{Pl-perc} \times A_{iv-ic} \qquad \text{(IV.E.1-1)}$$

## A-IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD

**Variable:** $DN_{DR}$ - Number of donations from an infected plasma donor, which varies based on type of plasma donated.

**Assumption used in the model:** We assumed individual infected Source Plasma donor most likely give - donations to a pool, with minimum of 1, maximum of 12 donations. Individual infected recovered plasma donors most likely give only one donation to a pool.

**Variable:** $I_{Pool}$- Initial infectivity in an infected plasma pool is represented by the equation:

$$I_{Pool} = I_{DN} \times DN_{DR} \qquad \text{(IV.E.2-1)}$$

## A-IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation

The FDA model employed three stratifications of clearance:

- $2-3\ \log_{10}$
- $4-6\ \log_{10}$
- $7-9\ \log_{10}$

Each of these levels of clearance was modeled separately. Most of the results are presented for the 4-6 $\log_{10}$ reduction during manufacture processing in the risk characterization section (Section V.) of this risk assessment.

**Assumptions used in the model:** The model assumed there are potentially three levels of reduction that may be achieved: a lower level of reduction (a range of 2 - 3 $\log_{10}$)-represented by uniform distribution (2, 3), medium level of reduction (a range of 4-6 logs, most likely, 5 $\log_{10}$)-represented by triangular distribution (4, 5, 6) and higher level of reduction (a range of 7-9 $\log_{10}$, most likely, $\log_{10}$)-represented by triangular distribution (7, 8, 9).

**Variable:** $I_{Pool}$- Initial infectivity in a specific infected plasma pool (calculated in A-IV. E.2)

**Variable:** $R_{Log}$- Potential log reduction in infectivity during processing

**Variable:** $I_{Pool-Ap}$- Remaining infectivity in a specific infected plasma pool after processing

$$I_{Pool-Ap} = I_{Pool} / 10^{R_{Log}} \qquad \text{(IV.F-1)}$$

**Variable:** $DR_{Pool}$- Size of plasma pool (number of donors/pool).

**Assumption used in the model:** The size of the plasma pools used in manufacturing was assumed to vary from pool to pool. In this risk assessment model, two different general distributions were used to represent frequency distribution of sizes of Source and recovered plasma pool based on the data provided by pdFVIII manufacturers.

**Data used in the model:** Information for Source Plasma pool size was collected by the FDA from pdFVIII manufacturers. The size of Source Plasma pools ranged from 6,000 donors per pool to 60,000

donors per pool with mean of ------ donations per pool. [FOI REVIEW] The distribution was generated based on the pool size data provided by pdFVIII manufacturers and the market share of the products based on information supplied annually to the FDA by manufacturers. Manufacturers supplied FDA with information on the average number of donations from individuals in the pool.

**Data used in the model:** Information for recovered plasma pool sizes was collected by the FDA from pdFVIII manufacturers. The size of recovered plasma pool ranged from 150,000 to 360,000 donations per pool. [FOI REVIEW] The distribution was generated based on the pool size data provided by pdFVIII manufacturers and the market share of the products. Manufacturers supplied FDA with information on the average number of donations from individuals in the pool.

**Variable:** $DN_{DR\text{-}Avg}$-Average number of donations from individual donors in the pool

**Assumption used in the model:** Data on the average number of donations per donor per pool were provided by manufacturers. We assumed the average number donations from individual donors varied from pool to pool. For Source Plasma, it was assumed to range from ------- donations per donor, with a most likely of ---- donations per donor. [FOI REVIEW] For recovered plasma, it was assumed that the most likely number of donations per donor was only 1.

**Variable:** $DN_V$-Volume of one unit of plasma, depending on plasma type (for Source Plasma, same as $DN_{V\text{-}S}$ used in A-IV. D. 2, recovered plasma, same as $DN_{V\text{-}R}$ used in A-IV. D. 2.)

**Variable:** $Y_{FVIII}$-Yield of pdFVIII (IU/L plasma)

**Assumption used in the model:** Based on the data provided by the World Federation of Hemophilia (2004) we assumed pdFVIII yield varies from pool to pool with minimum of 120, most likely of 187 and maximum of 250 IU per liter plasma.

**Variable:** $I_{iu}$- Quantity of infectivity in the pdFVIII product made from a specific infected pool (i.v. $ID_{50}$ per IU)

$$I_{IU} = (I_{Pool\text{-}Ap} / (DR_{Pool} \times DN_{DR\text{-}Avg} \times DN_V)) \times 1000 / Y_{FVIII} \qquad \text{(IV. F-2)}$$

## A-IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII

### A-IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients

This risk assessment provides outputs that estimate the annual exposure for several patient subpopulations with **Severe HA** disease for patients in the following clinical treatment groups:

- Prophylaxis – No inhibitor

- Prophylaxis - With inhibitor
- Prophylaxis - With inhibitor and immune tolerance
- Episodic – No inhibitor
- Episodic - With inhibitor

The study collected a total of 17,848 records, each record representing a single year of medical data for a single HA patient. The comprehensive study collected standardized information on patient demographics, clinical treatment and outcome data. Patient medical records were obtained from treatment sites including: hemophilia treatment centers (HTCs), hospitals, clinics, physician's offices, home-care agencies, nursing homes, prison infirmaries, and dispensers of factor concentrates. The data, abstracted from medical records, tabulated all recorded factor concentrate utilization prescribed by quantity, type, purpose (e.g., prophylaxis, treatment of acute bleeds, or immune tolerance therapy) and total quantity used per calendar year. Among all the records collected in the study from 1993-1998, 1,993 were from HA patients with severe disease that had been treated with human pdFVIII and the records were further grouped into five clinical treatment subcategories based on treatment regimen, including: prophylaxis, no inhibitor; prophylaxis,with inhibitor; prophylaxis, with inhibitor and immune tolerance; episodic, no inhibitor; and episodic, with inhibitor. Data from each of the five subpopulations were analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate initial descriptive statistics and distributions of pdFVIII usage by the HA patients. The data containing annual pdFVIII utilization information for patients in each of the five treatment groups were further analyzed using Best Fit software (Palisade Corp, New York) to generate a statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. Overall, the Generalized Beta distribution provided the most reasonable and consistent fit for the pdFVIII utilization data among all of the patient treatment groups. The Generalized Beta distributions were then used in the model to approximate the distribution of utilization of pdFVIII in each of the five HA patient subpopulations. FDA used the original patient data to not only generate statistical distributions for each patient treatment subpopulation. FDA also used the original data to identify the minimum and maximum dosages used by patients in each specific treatment subcategory and truncated each distribution using these values. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. We also provide a summary of the pdFVIII usage data from the CDC sponsored six state study, and also summarize the input Generalized Beta distributions generated with each subset of data in Table A-4.5.

**Table A-4.5. Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution**

| | | Original Data | | | Input distribution (Generalized Beta distribution) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment Regimen | Inhibitor Status | n | Mean | 95% CI | α | β | (min, max) | Mean | 95% CI |
| *Prophylaxis* | No Inhibitor | 578 | 164394 IU | (13574, 518781) | 1.5159 | 10.02 | (300, 1200000) | 157949 | (21242, 282316) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | With Inhibitor – No Immune Tolerance | 63 | 198781 | (7859, 937480 | 1.4640 | 6.2861 | (2000, 800000) | 190523 | (26956, 447639) |
| | With Inhibitor – With Immune Tolerance | 62 | 569707 | (14315, 3222471) | 0.8782 | 5.5081 | (100000, 2000000) | 558700 | (33235, 1592943) |
| *Episodic* | No Inhibitor | 946 | 90489 | (3001, 345416) | 0.9882 | 10.60 | (0, 1000000) | 85270 | (4633, 244656) |
| | With Inhibitor | 151 | 169710 | (4099, 835729) | 0.6950 | 3.6822 | (2200, 1000000) | 160458 | (5314, 488906) |

**Variable:** $IU_{Yr}$ - Annual usage of pdFVIII by individual HA patient of a specific clinical group (IU/yr, person)

**Variable:** $IU_{vial}$ - Vial size (IU/vial)

**Assumption used in the model:** We assumed there were equal numbers of vials for each of the four different package sizes (250, 500, 1000 and 1500 IU/vial) that are distributed in the US.

**Variable:** $Vial_{Tot}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

**Assumption used in the model:** We assumed individual patient uses pdFVIII products of the same package size throughout the whole year period of 2002 for which the model was run.

$$Vial_{Tot} = IU_{Yr} / IU_{Vial} \qquad \text{(IV.G. 1-1)}$$

**Variable:** $Pool$ - Annual number of plasma pool used to make pdFVIII (calculated in A-IV.D.2.b.)

**Variable:** $Pool_{vCJD}$ - Annual number of vCJD plasma pool used to make pdFVIII (calculated in A-IV.D.2.c.)

**Variable:** $Perc_{vCJD\text{-}vail}$ – Percentage pdFVIII vials containing vCJD agent

**Variable:** $Vial_{vCJD}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

$$Vial_{vCJD} = Vial_{Tot} \times Perc_{vCJD\text{-}vial} \qquad \text{(IV.G. 1-2)}$$

**Variable:** $I_{iu}$ - Quantity of infectivity in the pdFVIII product made from a specific infected pool (i.v. $ID_{50}$ per IU) (calculated in IV. F)

**Variable:** $I_{vr}$ - Annual exposure to vCJD through use of pdFVIII (i.v. $ID_{50}$/yr, person)

$$I_{yr} = \sum_{i=1}^{Vial_{vCJD}} I_{IU} \times IU_{Vial} \qquad \text{(IV.G. 1-3)}$$

## A-IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients

The CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 did not include patients with vWD. We assumed that vWD patients with severe disease would largely use Humate P product only for factor replacement treatment. A search of records in the Hemophilia Surveillance System project data revealed a total of 58 records that indicated Humate P had been used, among which, 8 records indicates patients had developed inhibitor, which are considered uncommon among vWD patients and were excluded from analysis. Among the 58 records, 35 were from Adults (≥15 yrs of age) and 23 records were from young persons (<15 yrs of age). Records for each age group were further grouped by clinical treatment using either a prophylaxis or episodic treatment regimen. Data were initially analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate descriptive statistics and statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. The Generalized Beta distribution was identified as the best fit to the pdFVIII utilization data (as determined by using the software Best Fit (Palisade Corp, NY) and was used as the input distribution for pdFVIII usage by individual vWD patients in the model. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. Table A-4.6. summarizes pdFVIII usage data from CDC sponsored study and the input distribution generated based on the data. FDA used data in the CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 to estimate FVIII utilization by all vWD patients. The data represent only a sample of all possible vWD patients with severe disease in the US. FDA estimated that there were approximately 250 patients in the US with Type 3 vWD. To calculate the total number of patients in each age group and treatment regimen group we adjusted the 58 patient population to equal a total of 250 patients by multiplying the patient population in each group by a factor of 4.3 (250/58 = ~4.3). The utilization data for patients in each treatment regimen in the sample population were used in the risk assessment model to generate outputs for the annual exposure to vCJD for all vWD for Adult (>15 yrs of age) and Young (≤15 yrs of age) persons in the US among clinical treatment groups of prophylaxis and episodic.

**Table A-4.6. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution We need to update the information in this table – based on new calculations for a total of 58 cases (previously it was 50 cases)**

| | Original Input Data | | | | Input Distribution (Generalized Beta distribution) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment Regimen | n | Percent of total population | Mean | 95% CI | α | β | (min, max) | Mean | 95% CI |
| Young (≤15 yrs of age) | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Prophylaxis* | 9 | 16% | 164193 | **(9200, 504625)** | 0.4523 | 0.9794 | (9200, 504625) | 165713 | (9346, 479457) |
| *Episodic* | 14 | 24% | 11122 | **(1010, 41850)** | 0.3900 | 1.1973 | (1010, 41850) | 11045 | (1013, 37543) |
| **Adult** (>15 yrs of age) | | | | | | | | | |
| Prophylaxis | 17 | 29% | 187538 | (15000, 772800) | 0.5741 | 1.9569 | (15000, 7728000) | 186880 | (15570, 606699) |
| Episodic | 18 | 31% | 845556 | (1000, 293800) | 0.5855 | 1.4097 | (1000, 293800) | 86923 | (1361, 260660) |

**Variable:** ***IUyr*** - Annual usage of pdFVIII by individual vWD patient of a specific clinical group (iu/yr, person)

**Variable:** $IU_{vial}$ - Vial size (IU/vial)

**Assumption used in the model:** We assumed that equal numbers of vials in each of three different package sizes (250, 500, 1000 IU/vial) are distributed on the market.

**Variable:** $Vial_{Tot}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

**Assumption used in the model:** We assumed individual patients used pdFVIII products of the same package size through out whole year period of 2002 for which the model was run.

$$Vial_{Tot} = IU_{Yr} / IU_{Vial} \qquad \text{(IV.G. 2-1)}$$

**Variable:** ***Pool*** - Annual number of plasma pool used to make pdFVIII (calculated in A-IV. D .2.b.).

**Variable:** $Pool_{vCJD}$ - Annual number of vCJD plasma pool used to make pdFVIII (calculated in A-IV.D.2.c.)

**Variable:** $Perc_{vCJD\text{-}vail}$ – Percentage pdFVIII vials containing vCJD agent

**Variable:** $Vial_{vCJD}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

$$Vial_{vCJD} = Vial_{Tot} \times Perc_{vCJD\text{-}vial} \qquad \text{(IV.G. 2-2)}$$

**Variable:** $I_{iu}$- Quantity of infectivity in the pdFVIII product made from a specific infected pool (iv. $ID_{50}$ per IU) (calculated in IV.F.)

**Variable:** $I_{yr}$- Annual exposure of individual vWD patients to vCJD through use of pdFVIII (i.v. $ID_{50}$/yr, person)

$$I_{yr} = \sum_{i=1}^{Vial_{vCJD}} I_{IU} \times IU_{Vial} \qquad \text{(IV.G. 2-3)}$$

# 医薬品　研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | － | | 研究報告の公表状況 | http://www.fda.gov/cber/blood/vcjdrisk.htm | 公表国：米国 | |
| 販売名（企業名） | － | | | | | |

**研究報告の概要**

FDA が米国内で承認されている血液凝固第 8 因子、第 9 因子およびその他の免疫グロブリンやアルブミンを含む血漿分画製剤のリスク評価を実施した。
その結果、米国で承認されている血液凝固第 8 因子製剤を投与されている患者の vCJD 発症リスクは極めて小さいとしており、第 9 因子などの他の血漿分画製剤についてはこれと同等か、さらに小さいとしている。
vCJD 病原体が存在する場合、他の血漿分画製剤、例えば第 9 因子、アルブミン、および免疫グロブリンなどが得られる血漿画分よりも、第 8 因子が得られる血漿画分には vCJD 病原体が多く含まれている可能性が高いため、FDA は第 8 因子のリスク評価を実施した。
第 8 因子を含む画分は、vCJD を減らしたり除去するための様々な方法を用いて製剤化される。また、vCJD を減らしたり除去したりできると考えられる方法は、他の血漿由来製剤の製造でも用いられている。
vCJD 発症リスクが最も高い英国で献血された血漿から製造された血液凝固因子製剤を大量に長期間にわたって投与されていた患者もいるが、FDA、CDC および NIH が把握する世界中の血友病患者、フォンウィルブランド病患者または他の血液凝固障害患者において、vCJD の発症が報告された症例はない。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 米国 FDA による血漿分画製剤の vCJD に対するリスク評価の情報で、現時点まで血漿分画製剤からの vCJD 伝播の報告はないと述べている。また、異常プリオン蛋白については、血漿分画製剤の製造工程で除去できるとの考え方がある。 | 今後とも vCJD に関する安全性情報等に留意していく。 |

**使用上の注意記載状況・その他参考事項等**

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

# Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD)

## From Plasma-Derived Products

In recent years, questions have been raised concerning the potential risk of variant Creutzfeldt-Jakob disease (vCJD - a rare but fatal brain infection) for recipients of plasma- derived clotting factors, including United States (US) licensed Factor Eight (pdFVIII), Factor Nine (pdFIX), and other plasma-derived products such as immune globulins and albumin. In response to these questions, FDA conducted a risk assessment. Based on the risk assessment, the US Public Health Service believes that the risk of vCJD to patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.

This web page provides FDA's risk assessment for US licensed pdFVIII and risk communication materials for this product and other plasma derivatives. Included are Key Points, and Questions and Answers. Additional links are provided to FDA's current guidance documents on deferral of blood and plasma donors who may be at increased risk of vCJD, and to other sources of information regarding vCJD.

**Documents Regarding US Licensed pdFVIII, and Other US Licensed Plasma Derivatives Including pdFIX**

- Potential vCJD Risk From US Licensed Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products: Summary Information, Key Points
- Risk Assessment (PDF, 582 KB)
- Risk Assessment Appendix (PDF, 623 KB)
- Questions and Answers on vCJD and pdFVIII
- Questions and Answers on vCJD and Plasma Derivatives Other than pdFVIII

**Guidance on Donor Deferral Related to CJD and vCJD**

- Draft Guidance for Industry: Amendment (Donor Deferral for Transfusion in France Since 1980) to "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" - 8/2006
- Questions and Answers on FDA Guidance: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob (CJD) Disease and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/22/2004
- Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/2002

**Other Sources of Information**

- Transmissible Spongiform Encephalopathies Advisory Committee
- Blood Products Advisory Committee Meeting – Summary of Recent TSEAC Meeting and Statement about FXI from the UK, on October 21, 2004
- Information on vCJD: Centers for Disease Control and Prevention
- Information on Bovine Spongiform Encephalopathy ("Mad Cow Disease"): US Department of Agriculture

**Patient Organizations:**

- Committee of Ten Thousand
- Hemophilia Federation of America
- National Hemophilia Foundation and/or HANDI
- World Federation of Hemophilia

Updated: March 15, 2007

# Potential Variant Creutzfeldt-Jakob Disease (vCJD) Risk

## From US Licensed Plasma-Derived Factor VIII (PdFVIII, Antihemophilic Factor) Products

**Summary Information**

**Key Points:**

- In recent years, questions have been raised concerning the risk of variant Creutzfeldt-Jakob disease (vCJD) (a rare but fatal brain infection) to hemophilia A and von Willebrand disease patients who receive US licensed plasma-derived Factor Eight (pdFVIII, Antihemophilic Factor) products.
- Based on a risk assessment, the US Public Health Service (PHS), including FDA, CDC, and NIH, believes that the risk of vCJD to hemophilia A and von Willebrand disease patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.
- Contacting a specialist in hemophilia or von Willebrand disease at a Hemophilia Treatment Center is a good way to learn about new information as it becomes available.

**Additional Information:**

- Between December 2003 and January 2007, there have been four reports of people, all in the UK, who probably acquired the vCJD agent through red blood cell transfusions. This has increased concern about the potential transmission of vCJD by blood products.
- Principal concerns are whether persons infected with vCJD could donate plasma in the U.S., and whether clotting factor products made from their plasma donations might transmit the disease.
- To address these concerns FDA recommends the deferral of donors who may have lived in or traveled extensively to countries with a higher prevalence of vCJD and bovine spongiform encephalopathy (BSE) than in the U.S.
- In the United States, pdFVIII products have not been made from the plasma of anyone known to have developed vCJD, and no one who received any of these products is known to have developed vCJD.
- FDA conducted a risk assessment for pdFVIII because the plasma fraction from which it is made is likely to contain more of the vCJD infectious agent, if present, than plasma fractions from which other plasma-derived products are made, such as Factor IX, (used to treat hemophilia B), albumin, and immune globulins. The FVIII-containing fraction is further processed using a variety of methods that are likely to reduce or potentially eliminate vCJD from the final pdFVIII product. Methods likely to reduce or potentially eliminate vCJD are also used in the manufacture of other plasma-derived products.
- FDA, CDC, and NIH are not aware of any cases of vCJD having been reported worldwide in patients with hemophilia, von Willebrand disease, or other blood clotting disorders. This includes those who have received, over a long period of time, large amounts of blood clotting factor products manufactured from plasma donations from the UK where the risk of vCJD is highest because of a previous higher risk of potential exposure to BSE- infected beef in the UK diet.
- The FDA has taken a number of steps to further reduce the potential vCJD risk from blood components. These steps include donor deferral recommendations, and quarantine and withdrawal of products at increased vCJD risk. Donor deferral guidance, first issued in August 1999 and subsequently updated, includes, among other things, deferral of donors who visited or resided in Europe where BSE prevalence is higher than in the US. Also, blood components and plasma derivatives are to be withdrawn if a donor is later diagnosed with vCJD. The potential spread of vCJD through red blood cell or plasma transfusion is limited by these deferral and quarantine measures that are in place.
- Additional steps FDA is taking to reduce potential vCJD risk from plasma derivatives include gathering, evaluating, and disseminating information about manufacturing processes that potentially could reduce the vCJD infectious agent in blood products. FDA is helping to develop donor screening and diagnostic tests for vCJD, and to inform patients and physicians about the current scientific understanding of vCJD risk from blood products.
- Using a computer model, FDA assessed the potential risk of vCJD infection from the current use of

pdFVIII products. However, because so much is unknown about vCJD and its prevalence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate the risk of vCJD in general, or of the actual risk to individual hemophilia A or von Willebrand disease patients. Meaningful distinctions also could not be made among specific products. There is no test yet available to detect vCJD infection in healthy donors or recipients.

- Although the risk of vCJD exposure from US pdFVIII products is most likely to be extremely small, it may not be zero, and FDA is encouraging physicians and patients to consider this risk, in the context of all remaining real or potential risks and the known benefits of product use, when making treatment decisions.
- At this time, the PHS does not believe there is a need for hemophilia A and von Willebrand disease patients who receive pdFVIII to inform their surgeons or dentists about their potential exposure to vCJD. Also, there is no recommendation for surgeons and dentists to take any special precautions based on such potential exposures. This belief is based on the results of the FDA risk assessment, as well as on the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK or anywhere else in the world. PHS agencies will continue to monitor and reevaluate the situation as new information becomes available.
- vCJD originally came from a disease in cattle called "mad cow disease" or BSE (bovine spongiform encephalopathy) . Transmission of the BSE agent to humans, leading to vCJD, is believed to occur primarily from eating beef and beef products contaminated with the BSE agent. Both BSE and vCJD are invariably fatal brain diseases with incubation periods typically measured in years.
- From 1995 through January 22, 2007, 201 individuals with vCJD were reported worldwide, with 165 in the United Kingdom (UK), and three in the United States. Two of the individuals in the United States had lived in the UK from 1980-1996 during a key exposure period to the BSE agent. The third individual most likely acquired the disease in Saudi Arabia. The reported incidence of vCJD in the UK based on disease onset peaked in 1999 and has been declining thereafter. In the UK, where most cases of vCJD have occurred, the current risk of acquiring vCJD from eating beef and beef products appears to be negligible.
- More information about vCJD is available on these government websites:
  - FDA: Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD) From Plasma-Derived Products
  - Centers for Disease Control and Prevention: vCJD (Variant Creutzfeldt-Jakob Disease)
  - US Department of Agriculture
- Information also may be obtained from these non-government sources:
  - Committee of Ten Thousand
  - Hemophilia Federation of America
  - National Hemophilia Foundation and/or HANDI
  - World Federation of Hemophilia

Updated: March 15, 2007

# Questions and Answers

## Variant Creutzfeldt-Jakob Disease (vCJD) and Plasma Derivatives Other than Factor VIII (pdFVIII)

**Q. What is the risk of vCJD for a patient who receives a US licensed plasma-derived product other than plasma-derived Factor VIII (pdFVIII)?**

**A.** The US Public Health Service, including FDA, CDC, and NIH, believes that vCJD risk from US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. We believe that the risk of other plasma derived products including plasma derived Factor IX, is likely to be as small as or smaller than for pdFVIII.

FDA conducted a risk assessment for pdFVIII because the plasma fraction from which it is made is likely to contain more of the vCJD infectious agent, if present, than plasma fractions from which other plasma-derived products are made, such as Factor IX, (used to treat hemophilia B), albumin, and immune globulins. The FVIII-containing fraction is further processed using a variety of methods that are likely to reduce or potentially eliminate vCJD from the final pdFVIII product. Methods likely to reduce or potentially eliminate vCJD are also used in the manufacture of other plasma-derived products, including Factor IX, albumin, and immune globulins.

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医薬部外品　　研究報告　調査報告書
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**研究報告の概要**

トピックⅠ：米国で認可されたヒト血漿由来第 VIII 凝固因子製剤（pdFVIII）の使用が関与する可能性がある vCJD についての FDA のリスク評価、及び公衆衛生局の考えられる対応

FDA は、血友病 A およびフォンウィルブランド病患者(vWD)によって使用される米国で認可された pdFVIII に係る vCJD リスク評価文書（Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States License From Plasma Collected in the US）を作成した。

リスク評価を行った背景には、2003 年以来、英国で輸血により vCJD に感染したと見られる 3 例が発見されたことがある。米国では BSE は 3 例発見されたのみで、米国で vCJD に感染したケースはまったくないため、血漿供与者が米国で生産された牛肉を食べて vCJD に感染するリスクは極度に低いと考えられる。しかし、米国の少数の供血者が英国、フランス、その他のヨーロッパ諸国への旅行や滞在で BSE に曝露された可能性はあり、従って一定のリスクを否定できない。これら供血者の一部は知らずに食事を通して vCJD に感染した可能性があり、米国で製造される pdFVIII に使われる血漿のプールにこれら感染者の血漿が入った恐れはある。供血停止政策で大部分の vCJD リスクは排除されている可能性が高いが、供血停止基準に合致しない供血者や、基準に合致していてもスクリーニングで排除されなかった供血者からのリスクは残存する。FDA はコンピュータシミュレーション・モデルを用い、pdFVIII 製品を利用した重症血友病 A 患者及び重症 vWD 患者について vCJD 病原体曝露の確度とそのレベルおよび vCJD 感染リスクを推定した。

FDA は、TSEAC にこのリスク評価文書を提示し、リスク評価の結果と解釈及びこの情報を医師、患者並びに一般大衆に知らせることに関する伝達の要点についての助言を求めようとしている。

トピックⅡ：pdFVIII での伝達性海綿状脳症（TSE）感染性実験のクリアランス

pdFVIII のリスクを、2-3、4-6 及び 7-9 の log クリアランス値を使って評価した。血友病 A の重症患者のリスクは、英国組織サーベイに基づく vCJD の高感染率を仮定した場合は年間平均 1/159～1/100,000,000、低感染率を仮定した場合は 1/21,500～1/3,200,000,000 と予想される。

FDA は委員会に対して以下の質問を行った。①製造工程をスケールダウンしスパイキングモデルを使った実験で示された TSE 病原体のミニマムのリダクション値によって、製品の vCJD 安全性が強化されるか？②もし委員会が vCJD 安全性を強化するミニマムの TSE リダクションを定めた場合、承認を受けた pdFVIII のリダクション値がそれより低い場合に FDA は如何なる対応をしなければならないか？

**使用上の注意記載状況・その他参考事項等**

代表として静注用ヘプスブリン－IH の記載を示す。

2. 重要な基本的注意

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2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 2006 年 12 月 15 日開催の米国海綿状脳症委員会の議題であり、資料の中に血液凝固第Ⅷ因子製剤の vCJD についての FDA のリスク評価案及び公衆衛生局が医師や患者に事実を如何に伝えるかについての案が示されている。(BENE2006-031 が審議結果の報告)<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

# TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES ADVISORY COMMITTEE MEETING
**December 15, 2006**

## ISSUE SUMMARY

**Topic I:** **FDA's Risk Assessment for Variant Creutzfeldt- Jakob Disease (vCJD) Potentially Associated with the Use of US Licensed Human Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products, and Potential Public Health Service Responses**

**Issue:** **FDA has prepared a risk assessment of the potential, but unproven, risk related to pdFVIII products, which are used by some patients with the blood clotting disorders hemophilia A and von Willebrand disease. The risk of the potential of pdFVIII products to transmit vCJD, the agent which causes the human form of "Mad Cow Disease," is highly uncertain, but appears likely to be very low. FDA is presenting the risk assessment to the TSEAC to seek advice on key message points concerning both the risk assessment and appropriate ways to communicate this information to physicians, patients, and the general public. Prior to the TSEAC meeting, FDA has obtained input on the risk communication from individual special government employees (SGE's) who represent various hemophilia advocacy organizations.**

**BACKGROUND:**

vCJD is a fatal neurodegenerative disease acquired through infection with the agent that causes bovine spongiform encephalopathy (BSE) by consumption of beef products from infected cattle. The first human cases of vCJD were reported in the United Kingdom (UK) in 1996, and as of August 2006, 195 cases have been reported worldwide, 162 of them in the UK.

In 1999, based on the potential, but unknown risk of vCJD from blood products, and consistent with advice from TSEAC, FDA recommended deferral of blood and plasma donors who had traveled or lived for 6 months or longer in the UK from the presumed start of the BSE outbreak in the UK in 1980 until the end of 1996 when the UK had fully implemented a full range of measures to protect animal feed and human food from contamination with the infectious agent causing BSE. In January 2002, FDA recommended enhancing the vCJD geographical donor deferral policy by reducing the time that an otherwise suitable blood donor might have spent in the UK from six to three months. FDA also recommended deferring donors who had spent five or more years in France or cumulatively in any European country listed by the USDA as either having had BSE or having a significant risk of BSE, and adding certain other measures to reduce potential risk, such as deferring any donor with a history of blood transfusion in the UK after 1979. Taken together, these steps were estimated to have excluded donors representing slightly more than 90% of the potential BSE/vCJD risk while deferring about 7% of otherwise suitable donors. Since 2002, TSEAC has several times reviewed FDA vCJD/CJD blood donor deferral policies, most recently advising FDA to recommend deferral of blood donors transfused in France. FDA has issued draft guidance containing such recommendations..

Since 2003, three presumptive transfusion transmitted cases of vCJD have been reported in the UK. Two of the transfusion recipients died of vCJD, whereas the third had vCJD infection detected after death from an unrelated cause. Each had received transfusions of red blood cells from individuals who were asymptomatic at the time of donation, but who later died from vCJD. The strong likelihood that transmission of vCJD occurred via transfusion of red blood cells in the UK increased the concern that products manufactured from the plasma portion of human blood might also pose a risk of vCJD transmission. Note, however, there have been no reported cases of vCJD in any recipients of plasma derived products in the UK, where risk is considered greatest, or anywhere else in the world. In the fall of 2004 UK authorities notified recipients of a number of plasma derivatives that they might be at increased risk of vCJD. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

Because BSE occurs at an extremely low level in US cattle (only three reported cases: two in US born cattle and one in a cow imported from Canada), and there have been no human cases of vCJD acquired in the US, any risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be extremely low. However, it is possible that a small population of US donors may have been exposed to the BSE agent during travel or residence in the UK, France, or other countries of Europe and may therefore be at some risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through dietary exposure and then contributed donations to plasma pools used to manufacture pdFVIII in the U.S. The donor deferral policy likely removes most of the vCJD risk; however, there may be residual risk in the US donor population for persons who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process.

FDA has developed a computer-based simulation model to try to estimate the potential risk and understand factors involved in determining and potentially reducing the degree of risk. The model and various risk estimate results and uncertainties are presented in the document "A Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US" provided as a separate attachment. The results are estimates of the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and the severe form of von Willebrand disease (vWD) (Type 3) who have used human pdFVIII product manufactured in the US.

The parameters used in this risk assessment were derived after receiving recommendations from the TSEAC about another risk assessment, the "Draft Risk Assessment: Potential Exposure to the vCJD agent in United States Recipients of Factor XI Coagulation Product Manufactured in the United Kingdom" at the February 8, 2005 meeting; from discussion at a meeting of the TSEAC on October 31, 2005 on several risk assessment model inputs for plasma derivatives and potential vCJD risks specifically related to pdFVIII; and from peer review by non-FDA experts in risk assessment.

The ranges of uncertainty and variability in the input parameters of the risk assessment are great, resulting in very large uncertainty in the model's outputs that estimate potential risk. Additionally, because scientific data on the level of exposure to the vCJD agent and the

likelihood of certain human health outcomes, such as infection and illness, are lacking, estimates for the infectious risk generated in the risk assessment may not be accurate. For these reasons, it is not possible to provide an actual estimate of the vCJD risk to individual patients potentially exposed to the vCJD agent through plasma-derived products. Additionally, the absence of any reported cases of vCJD in repeatedly exposed recipients of plasma derivatives in the UK, where risk is thought to have been greatest, or anywhere else in the world, further suggests that the actual risk of vCJD from pdFVIII is likely to be very low.

Nevertheless, FDA believes it is appropriate to share both the findings of and the uncertainties in our risk assessment for pdFVIII with physicians, patients, and the general public since it is possible that the risk is not zero. Advice will be sought from the TSEAC in December concerning communication of the findings of the risk assessment and its interpretation, given the very wide range of uncertainty in the estimate of vCJD risk. Advice will also be sought on potential steps that may help to better understand as well as to further reduce any risks.

**DISCUSSION:**

**A. Risk Assessment and Interpretation**

The FDA risk assessment estimates the risk of vCJD infection as a function of product exposure for different assumed levels of vCJD infectious agent clearance in manufacturing of pdFVIII and under two assumed levels of vCJD prevalence in the UK. The risk assessment utilizes a probability-based statistical sampling model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII beginning with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various representative patient subpopulations. Input data for parameters used in the model such as clearance, vCJD prevalence, and pdFVIII usage are represented as statistical distributions that express the underlying uncertainty and variability. Each run of the model randomly samples one number from the distribution for each parameter; this is done thousands of times to generate a single distribution representing the final risk estimate that expresses, where possible, the accompanying uncertainty of these risk estimates. A sensitivity analysis, conducted by varying values of key parameters within the input range of the model and observing the effect on the predicted outcomes, determined that three major factors in the model greatly influenced potential vCJD risk: reduction of the infectious agent by the manufacturing process, intensity of pdFVIII utilization by the patient, and differing estimates of disease prevalence in the UK.

One of the most influential risk assessment parameters for vCJD risk is the manufacturing process, which may reduce or eliminate the amount of the vCJD agent in the final product. Because of the uncertainty and variability in the levels of vCJD clearance afforded during the manufacturing process for any pdFVIII product, the model evaluated three separate categories of reduction in infectivity that the product may have undergone during manufacturing including 2-3 $\log_{10}$, 4-6 $\log_{10}$, and 7-9 $\log_{10}$ reduction. These three categories are meant to span the possible range of uncertainty and variability in reduction of vCJD agent for marketed pdFVIII products. Based on currently available experimental studies, FDA believes that all US licensed pdFVIII products probably achieve at least a 4 $\log_{10}$ of vCJD level of clearance during manufacture.

Another major variable affecting potential risk is the quantity of product used by patients in different treatment groups. For purposes of this model only patients with severe HA were considered because their higher use of product would put them at higher risk than patients with mild or moderate forms of the disease. Severe HA patients account for approximately 50% of the total HA population. Approximately 25% of all HA patients use pdFVIII products, while most others use recombinant FVIII. Data from a CDC sponsored epidemiological study on HA patients were used to generate the statistical distribution of pdFVIII usage by patients. Using these estimates, the risk assessment evaluated different treatment regimens including patients on prophylaxis, prophylaxis plus inhibitor, prophylaxis plus inhibitor plus immune tolerance, episodic treatment, and episodic treatment plus inhibitor. It also evaluated the potential of patients with severe von Willebrand disease (vWD) to be exposed to the risk of infection with vCJD through the use of pdFVIII. These patients must use plasma-derived sources of FVIII/von Willebrand factor because there is presently no recombinant von Willebrand factor.

The prevalence of vCJD in the United Kingdom population was used to estimate vCJD exposure and therefore prevalence in US donors who traveled to the UK, France and other European countries. For purposes of the risk assessment, we used two different estimates of vCJD prevalence in the UK. One estimate is based on epidemiological modeling of actual reported cases and an estimate of future vCJD cases in the UK. The other estimate is based on testing for prion protein in a relatively small tissue (tonsil and appendix) surveillance study. At present it is not possible to know which of the two estimates of vCJD prevalence in the UK provides a better estimate of the true prevalence.

The vCJD case prevalence based on epidemiological modeling of actual reported cases and an estimate of future vCJD cases in the UK yielded an estimate of approximately 1.8 vCJD cases per million. There are a number of limitations to this estimate based on simplifying assumptions that contribute to considerable uncertainty in the final case estimates. These assumptions include the intensity of human exposure to the BSE agent, the length of the incubation period, and how a person's age and genetic background could influence exposure, disease susceptibility, and incubation period. However, it is possible that the prevalence of vCJD in the UK is higher than this estimate if there are people infected who never develop the disease (but can still spread the infection) or if some individuals take an extremely long time to become ill. Therefore, we also used a second approach to estimate vCJD infection prevalence based on testing for prion protein in a relatively small tissue (tonsil and appendix) surveillance study. This approach yielded an estimate of 1 in 4,225 (237 infections per million). Limitations to this study that contribute to uncertainty include the fact that this study was not controlled using tissues from a non-BSE exposed population and false positive findings cannot be ruled out. It is also not known whether this finding of prion protein in tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation.

After accounting for the age distribution, incubation period, country, year and duration of travel, these two different prevalence estimates have been used to predict the number of vCJD donations that could be present in variably sized pools of US donated plasma.

## Results

See Risk Assessment, including the Executive Summary, and especially Tables I.A and II.A in the Executive Summary and attached here in an Appendix.

**Interpretation**

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Donor deferral criteria in place since 1999 have reduced the risk of donation by exposed persons.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the modeling predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from 1 in 15,000 for the higher vCJD prevalence estimate and high product usage, to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. While higher levels of clearance are likely to reduce risk, it is not possible at this time to determine with certainty if a specific product may be less or more safe than another due to the wide range of methods used for clearance studies currently available, the results themselves, and gaps in information. Although results of the model suggest that exposure to vCJD agent could occur, and that there is a potential risk of infection that is likely to be generally very low, it is not possible for the model to provide a precise estimate of the vCJD risk in general, or of the actual risk to individual patients. Despite the uncertainties in the model, we believe this is information that patients and physicians might consider when making treatment decisions.

**B. <u>Risk Management Strategy, Key Message Points, and Communication Strategy</u>**

**Risk Management Strategy**

FDA's current risk management strategy for vCJD has evolved in response to emerging epidemiologic findings and basic scientific developments pertinent to the epidemic. The overall risk management strategy for vCJD includes:

- Deferral of donors at increased risk of vCJD based on epidemiological data, and withdrawal of products at increased vCJD risk
    - Donor deferrals: Guidance since August 1999 (most recently updated in January 2002) to defer donors with "geographic risk," e.g. that visited or resided in countries where BSE prevalence is higher; deferral of donors that used UK-sourced bovine insulin; deferral of donors transfused in the UK since 1980 (note also draft guidance published in August 2006 for deferral of donors transfused in France since 1980).
    - Withdrawal of implicated blood components and plasma derivatives is recommended if a donor is diagnosed with vCJD

- Facilitating development, validation, and information sharing (including product labeling) regarding the performance of manufacturing processes in clearance of TSE agents from blood products.
    - FDA reviews requests for TSE clearance labeling claims which may be approved if detailed, validated TSE clearance study data are provided
    - On September 18, 2006, FDA discussed with TSEAC the feasibility and scientific value of standardized assessments of TSE clearance in the manufacturing processes for pdFVIII. The topic will be addressed again at this meeting
- Facilitating development of candidate donor screening and diagnostic tests for vCJD and other TSEs
    - FDA has held meetings with candidate test kit manufacturers to discuss developmental pathways
    - A public discussion of validation for donor screening tests for vCJD and other TSEs was discussed with the TSEAC on September 19, 2006
- Risk assessment and communication to inform patients and physicians about the current scientific understanding regarding vCJD risk from blood products and to help in informing treatment decisions
    - FDA has engaged in periodic reassessment of TSE epidemiology and pathogenesis to determine whether guidance/policies need to be revisited in light of new information
    - FDA performed risk assessments for potential exposure to vCJD in investigational pdFXI made from plasma donated in the UK, and for pdFVIII made from plasma donated in the US

The risk assessments that FDA has performed for potential exposure to vCJD from licensed pdFVIII made from US plasma has led us to consider further actions. Based on the finding that, despite very large uncertainties in the risk assessment models and generally low risk estimates, the risk of vCJD exposure from these products may not be zero, and we are taking the following steps:

- The risk assessment model has indicated that the level of TSE agent clearance during product manufacturing is one of the most important parameters for vCJD risk. For this reason, FDA seeks to improve our understanding of the actual prion clearance in manufacturing by encouraging standardized clearance studies of the different pdFVIII products, and, potentially, by setting minimum standards for TSE agent clearance.
- Although the risk of vCJD exposure from US pdFVIII products is likely to be very low, and it may not be zero, and FDA is encouraging physicians and patients to consider this risk in making treatment decisions.

**DRAFT Risk Communication Messages**

Based on the findings of the risk assessment FDA has developed key message points and additional, background information about the risk of vCJD in pdFVIII made from US donor plasma. They include the following:

Key Messages

vCJD (variant Creutzfeldt Jakob Disease, the agent which causes the human form of "Mad Cow Disease") is rare, even in the UK. Infection is not known to have been acquired in the US and there is no evidence that any US plasma donor has had vCJD. FDA has put steps in place to reduce the risk of transmission by deferring plasma donation from individuals with the most potential risk of exposure, those who lived in affected areas for significant time periods. Exposure to vCJD through pdFVIII could still potentially occur if someone traveled or resided briefly in the UK or another area where TSE is present, and became infected without knowing it from eating infected meat, and then donated plasma. While blood transfusion has transmitted vCJD in the UK, pdFVIII products go through additional manufacturing steps likely to help remove the agent and reduce or eliminate the risk of transmitting the disease.

FDA used a computer risk assessment model to help estimate any remaining potential risk to US pdFVIII recipients. While there are still many uncertainties, and it is not possible to estimate risk for specific individuals, the model suggests that any potential risk to the health of most US pdFVIII recipients is likely to be very low. Also, no cases of vCJD have yet been reported in patients receiving such products either in the UK, where the risk is highest, or anywhere else in the world.

Because so much is unknown about vCJD and its incidence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate any risk. However, we wanted to provide information about the risk assessment so that patients and health care providers can consider the matter, where appropriate, in their treatment decisions.

Additional Information

vCJD is a very rare, fatal disease that can infect a person for many years before making people sick by destroying cells in the brain.

Most cases of vCJD have occurred in the UK and individuals there are thought to be at higher risk for the disease than individuals elsewhere.

Beef products contaminated with the infectious agent of BSE are the main cause of vCJD. As of August 2006, world-wide, 195 cases have been reported, 162 of them in the UK. Food risk in the US blood donor population is expected to be very low as there have been only three cases of BSE found in US cattle (two US born, and one imported from Canada) and safeguards are in place to prevent infected beef products, if present, from entering human food.

While most vCJD is due to eating infected beef products, there is convincing evidence that the disease has also been transmitted by red blood cell transfusion. In

the UK, three people who became infected with the vCJD agent had received blood from donors who later developed vCJD

Plasma is the liquid part of blood, after the cells are removed, that is used for manufacture of plasma-derived products such as pdFVIII. Animal studies show that when blood carries this infection, so does the unprocessed plasma.

Manufacturing steps used in making most pdFVIII products appear likely to be effective in removing the agent and may reduce or eliminate most risk even if a vCJD infected donor contributed plasma.

Because so much is unknown about vCJD and its incidence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate any risk. The risk assessment model suggests that important contributors to risk are how common vCJD is in the donor population, the degree to which the manufacturing process can remove the agent from the products, and the quantity of product that individuals use.

The Public Health Service believes the risk of developing vCJD infection from pdFVIII is likely to be very low, given both the results of the risk assessment and the lack of any reported cases of vCJD in plasma-derived blood products following decades of use, including in the UK, where the risk is considered greatest. For example, for the most common pattern of use (i.e. episodic, no inhibitor) of a pdFVIII product made using processes with a level of clearance believed to be achieved by most manufacturing processes, the model suggests a possible estimated risk of from 1/105,000 to 1/9.4 million infections per person per year, depending on which prevalence estimate is assumed, or a possible total of 1 case in the population of severe HA patients using such products every 35 to 3,077 years. However, there is a great deal of uncertainty in the model, including how effective clearance may be, and it is also possible that not enough time has passed for some people receiving plasma products that contained the vCJD agent to have developed signs of infection. Therefore, while the risk is estimated to be very low, it may not be zero.

While there is no proof of a significant risk at this time, patients and physicians utilizing pdFVIII should be aware of the possibility and consider both the potential risks and benefits of their treatment.

Efforts to better understand and reduce any potential risk of transmission of vCJD by plasma products are ongoing. PHS will provide additional information as it becomes available.

**Communication Strategy**

Subject to further input from the PHS agencies and possible revisions, the Key Messages and Additional Information cited above are the messages that the FDA, in cooperation with other components of the Public Health Service would utilize for communications with the patient community and health care providers, through various media presentations including government web sites, press, and communications with Hemophilia Treatment Centers and consumer organizations.

# APPENDIX

**Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent:** *Predicted mean potential per person annual vCJD risk using two different UK vCJD prevalence estimates.*

| | | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|---|
| | | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| **Treatment Regimen** | **Inhibitor Status** | **Est. Total Number patients in US** | **Mean quantity FVIII used per person per year (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** |
| ***Prophylaxis*** | No Inhibitor | 578 | **157949 IU[c]** (21242 , 382316 ) | 1 in 4.0 million (0-0)[d] | 1 in 54,000 (0 - 1 in 12,000) |
| | With Inhibitor – No Immune Tolerance | 63 | **190523 IU[c]** (26956 , 447639) | 1 in 4.8 million (0-0)[d] | 1 in 41,000 (0 - 1 in 9,000) |
| | With Inhibitor – With Immune Tolerance | 62 | **558700 IU[c]** ( 33235, 1592943) | 1 in 1.3 million (0-0)[d] | 1 in 15,000 (0 - 1 in 3,700 ) |
| ***Episodic*** | No Inhibitor | 946 | **85270 IU[c]** ( 4633, 244656) | 1 in 9.4 million (0-0)[d] | 1 in 105,000 (0 - 1 in 24,000 ) |
| | With Inhibitor | 151 | **160458 IU[c]** (5314 , 488906 ) | 1 in 8.0 million (0-0)[d] | 1 in 48,000 (0 - 1 in 12,000 ) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[d] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

**Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk:**

- Assuming a reduction from manufacturing of 4-6 log $_{10}$, and
- Two different UK vCJD prevalence estimates.

| | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | *Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)* |
| | Est. Total Number patients in US | Mean quantity product used per person per year | Mean vCJD risk per person per year[b] (5th - 95th perc)[c] | Mean vCJD risk per person per year[b] (5th - 95th perc)[c] |
| **YOUNG vWD (≤ 15 yrs of age)** | | | | |
| *Prophylaxis* | 39 | 165,713 IU[d] (9876, 454306) | 1 in 4.7 million (0-0)[e] | 1 in 52,000 (0 - 1 in 13,000) |
| *Episodic* | 60 | 11,045 IU[d] (1025, 34352) | 1 in 48 million (0-0)[e] | 1 in 971,000 (0 - 1 in 293,000) |
| **ADULT vWD ( > 15 yrs of age)** | | | | |
| *Prophylaxis* | 73 | 186,880 IU[d] (16910, 539877) | 1 in 4.1 million (0-0)[e] | 1 in 46,300 (0 - 1 in 11,000) |
| *Episodic* | 78 | 86,923 IU[d] (2182, 240338) | 1 in 10 million (0-0)[e] | 1 in 1 million (0 - 1 in 24,000) |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

# DRAFT

# Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US

October 15, 2006

Center for Biologics Evaluation and Research
US Food and Drug Administration

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# TABLE OF CONTENTS

EXECUTIVE SUMMARY.....1
RISK ASSESSMENT
I. INTRODUCTION.....9
II. HAZARD IDENTIFICATION.....12
III. HAZARD CHARACTERIZATION.....16
IV. EXPOSURE ASSESSMENT.....17
IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1).....20
IV. A. 1. UK vCJD prevalence estimated using epidemiological modeling results (Clarke and Ghani 2005) and diagnosed vCJD cases for 2002 and 2003.....21
IV. A. 2. UK vCJD Prevalence derived from a Tissue Surveillance study.....23
IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2).....24
IV. C. Estimation of the probability that a plasma pool may contain a donation that contains vCJD agent.....27
IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood...28
IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in their blood and who may not be deferred by questionnaire screening.....34
IV. D. Annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US.....38
IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD.....39
IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD.....40
IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD.....41
IV.E. 3. Model results: Estimates of the per vial vCJD infection risk for US manufactured pdFVIII (Module 3).....43
IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation.....45
IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII.....47
IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients.....47
IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients.....48
V. RISK CHARACTERIZATION.....50
V.A. THE MODEL.....50
V. B. Model results: Estimated annual potential exposure to vCJD i.v. $ID_{50}$ and potential vCJD risk through human pdFVIII used to treat severe HA.....51
V. C. Model results: Estimated annual potential exposure to i.v. $ID_{50}$ vCJD agent and potential vCJD risk through human pdFVIII used to treat severe von Willebrand disease (vWD).....55

V. D. Sensitivity analysis.....62
V. E. Uncertainty and Data Gaps.....66
V. F. Conclusions.....68

REFERENCES .....70

# TABLE OF TABLES

## EXECUTIVE SUMMARY

Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent .....4
Table I.B. Model Results for Mean Total Population-based Potential vCJD Risk for all Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....5
Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk.....6
Table II.B. Von Willebrand Disease (vWD) patients[a] with Severe Disease: Predicted Total Population-based Potential vCJD Risk.....7

## RISK ASSESSMENT

Table 4.1. FDA model estimation of UK vCJD cases for years 2002 – 2080.....22
Table 4.2. Summary of surveillance testing of tonsil and appendix tissues in the UK.....23
Table 4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups .....26
Table 4.4 Model Results: Annual Number of US plasma donors predicted by model to be potentially infected with vCJD and donate to plasma pools used to manufacture pdFVIII.....37
Table 4.5 Annual Percentage of US Plasma Pools Potentially containing a vCJD Donation.....39
Table 4.6 Reduction factor (RF) of fractionation procedures.....43
Table 4.7 Annual Predicted per Vial vCJD Infection Risk for US Manufactured pdFVIII from Model.....44
Table 4.8 Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution.....48
Table 4.9. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution..49
Table 5.1A. Model Results for All HA Patients who use a Hypothetical Factor VIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....53
Table 5.1B. Model Results for Total Population-based Exposure and Potential vCJD Risk for All Hemophilia A patients who use a Hypothetical pdFVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent.....54
Table 5.2A. Results von Willebrand Disease (vWD) patients[1] with Severe Disease: Predicted Potential Annual Exposure to vCJD i.v. $ID_{50}$ and vCJD Risk.....55
Table 5.2B. Von Willebrand Disease (vWD) Patients[1] with Severe Disease: Predicted Total Population-based Exposure to vCJD i.v. $ID_{50}$ and Potential vCJD Risk.....57
Table 5.3A. Range of Predicted Annual Mean Potential per HA Patient vCJD risk for pdFVIII.....60
Table 5.3B. Range of Total Population-based Exposure and Potential vCJD Risk from Model .....61
Table 5.4. Input Variables included in Importance Analysis .....63

# TABLE OF FIGURES

Figure 1. Exposure Assessment diagram.....19
Fig 2. A. Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using prevalence estimate encompassing the range of values for both high and low prevalence from 0.7 to 700 vCJD cases per million UK population.....64
Fig 2. B. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Tissue Surveillance-based (HIGH) prevalence estimate.....65
Fig 2. C. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Epi Modeling-based (LOW) prevalence estimate. Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.....65

# EXECUTIVE SUMMARY

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the U.K. in 1996 – and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the U.K. Since December 2003, there have also been three reports in the United Kingdom (U.K.) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness.

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the U.K., where the risk is considered greatest, or elsewhere in the world. U.K. authorities have notified physicians in the U.K. and their patients who received plasma derivatives made from plasma from U.K. donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

This document "Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US" quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe disease who have used human plasma-derived Factor VIII (pdFVIII) product manufactured in the US. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may have been exposed to the BSE agent during extended travel or residence in the UK, France or other European countries and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. Risk for these products was estimated for the baseline year of 2002 but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. A few major elements of the model greatly influence vCJD risk. The most influential of these are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the risk estimate is significantly affected by the prevalence

of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe. The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

Patients with hemophilia A (HA) have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a glycoprotein circulating in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or within a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with vWD (Type 3) have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII coagulant activity (anti-hemophilic factor) as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually require treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

**Results from the Model**

An important, yet also highly uncertain parameter in driving the risk assessment results is the estimate used for vCJD prevalence in the UK. The prevalence of vCJD in the UK population was estimated in the model using two different approaches. The first approach to estimating vCJD prevalence in the UK was from a study based on epidemiological modeling that was derived using actual reported vCJD cases in the UK combined with an estimate of future vCJD cases (Clarke and Ghani, 2005). Several factors used in epidemiologic modeling approaches are difficult to quantify and add uncertainty to the final estimated number of future vCJD cases. These factors include: the intensity of human exposure to the BSE agent, incubation period, time of infection, and whether illness will develop in individuals who are not homozygous for methionine at codon 129 of PrP. All cases of vCJD to date have occurred in individuals who are homozygous for methionine at this location. Our calculations, based on the Clarke and Ghani study (2005) and diagnosed cases in 2002 and 2003, yielded a prevalence estimate of approximately 1.8 vCJD cases per million in the UK.

Running the model with this vCJD case prevalence estimate (~1.8 per million) produces an estimate suggesting that, on average, there was a 0.027% likelihood that a plasma pool, which then undergoes manufacturing, will contain at least one donation from an individual whose blood contains the vCJD agent. Therefore, on average, more than 99% of the time the model predicts the product as administered will contain no vCJD agent and this is reflected in the (0 – 0) values for the 5$^{th}$ and 95$^{th}$ percentiles shown for the lower prevalence estimate results in Table I.A. (below).

However, it is possible that the prevalence of vCJD in the UK is higher than that estimated above. This could happen if there are people infected who never develop the disease (but can still spread the infection) or if some individuals take extremely long to become ill. Therefore, a second approach to estimating vCJD infection prevalence was used based on a relatively small tissue surveillance study by Hilton, *et al* (2004), which tested stored tonsil and appendix tissues from the UK for accumulation of abnormal prion protein. It yielded a much higher prevalence estimate of 1 in 4,225 (237 infections per million). This study was not controlled using tissues from a non-BSE exposed population and false positive findings cannot be ruled out. It is also not known whether this staining of appendiceal tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate that may be relevant to transfusion risk and therefore should also be considered. Use of these data as the basis for a vCJD infection prevalence estimate which is then used in the model produces a significantly higher estimate suggesting that, on average, if it were correct, there could be a 2.41% likelihood that a plasma pool, which then undergoes manufacturing, may contain at least one donation from an individual whose blood contains the vCJD agent.

### *Estimated annual potential vCJD risk associated with human pdFVIII used to treat severe Hemophilia A*

Results from the model indicate that it is possible that a donor unknowingly infected with vCJD may have donated plasma used in the manufacture of pdFVIII in the US. Output from the model using the lower UK vCJD prevalence estimate (~1.8 in 1 million) indicated that, on average, there is a 0.027% (95% CI: 0 % - 0 % ) likelihood that a plasma pool may contain at least one donation from an individual with the vCJD agent in their blood. Readers may notice that the 5$^{th}$ and 95$^{th}$ percentile intervals for all of the model outputs are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. This means that at least ninety five percent of the time the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, actual model predictions indicated that, at the lower prevalence, 0.027% of the time the exposure to vCJD may be greater than zero. When the model was run using the higher UK vCJD prevalence estimate (1 in 4,225) to derive an estimate for vCJD prevalence in US plasma donors, the FDA model predicted that, on average, there is an approximately 2.41% (95% CI: 0 % - 10 %) likelihood that a plasma pool will contain at least one donation from an individual with the vCJD agent in their blood. For either set of results, the model assumes that if vCJD agent were present, the amount in a plasma pool would likely be reduced or possibly eliminated by processing steps used during the manufacture of pdFVIII product.

Individuals with HA vary in their degree of FVIII deficiency. For simplicity, the model results and this executive summary specifically address potential vCJD exposure and risk for persons with severe HA. FDA estimates that among the total population of 14,000 HA patients in the United States, approximately 1,800 (Table I.A.) have severe disease and use pdFVIII products. FDA obtained data

on FVIII utilization from the Centers for Disease Control (CDC). The data were generated as part of a collaborative effort between CDC and six states in a study conducted from 1993 –1998. Treatment regimens for HA are administered either as prophylaxis to prevent the occurrence of bleeding episodes or on an episodic basis to control bleeding when it occurs. Additionally, inhibitors may be treated with very high doses of pdFVIII to induce immune tolerance. Assuming these patients are treated with a pdFVIII product that has a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent, Table I.A. displays model outcomes for patients treated using either prophylaxis or episodic treatment, and with respect to their inhibitor status.

**Table I.A. Model Results for all Severe Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $\log_{10}$ Manufacture Process Reduction of vCJD Agent:** *Predicted mean potential per person annual vCJD risk using two different UK vCJD prevalence estimates.*

| | | | | 4 - 6 $\log_{10}$ Reduction | |
|---|---|---|---|---|---|
| | | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*** |
| **Treatment Regimen** | **Inhibitor Status** | **Est. Total Number patients in US** | **Mean quantity FVIII used per person per year (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** | **Mean potential vCJD risk per person per year[a] (5th - 95th perc)[b]** |
| ***Prophylaxis*** | No Inhibitor | 578 | 157949 IU[c] (21242 , 382316 ) | 1 in 4.0 million (0-0)[d] | 1 in 54,000 (0 - 1 in 12,000) |
| | With Inhibitor – No Immune Tolerance | 63 | 190523 IU[c] (26956 , 447639) | 1 in 4.8 million (0-0)[d] | 1 in 41,000 (0 - 1 in 9,000) |
| | With Inhibitor – With Immune Tolerance | 62 | 558700 IU[c] ( 33235, 1592943) | 1 in 1.3 million (0-0)[d] | 1 in 15,000 (0 - 1 in 3,700 ) |
| ***Episodic*** | No Inhibitor | 946 | 85270 IU[c] ( 4633, 244656) | 1 in 9.4 million (0-0)[d] | 1 in 105,000 (0 - 1 in 24,000 ) |
| | With Inhibitor | 151 | 160458 IU[c] (5314 , 488906 ) | 1 in 8.0 million (0-0)[d] | 1 in 48,000 (0 - 1 in 12,000 ) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[d] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

The risk estimate for the entire severe HA population of 1,800 in the US who use pdFVIII, obtained by summing the total annual exposure and vCJD risk, is shown in Table I.B. Variant CJD risk for US donors with a history of travel to the UK, France or other countries in Europe since 1980 is further adjusted to account for donor age, country, duration and year of travel. Using the lower UK prevalence estimate as a starting point, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 3,077 years of treatment. If the higher vCJD prevalence estimate is used, the model estimates that the total patient population may be exposed to a potential population-based vCJD risk of 1 case observed in 35 years of treatment.

**Table I.B. Model Results for Mean Total Population-based Potential vCJD Risk for all Hemophilia A Patients who use a Hypothetical Plasma-derived FVIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent.** Risk estimates were calculated for patients with severe disease, using two different UK vCJD prevalence estimates.

| | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*** |
| | **Est. Total Number severe HA patients in US** | **Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c]** | **Mean population –based potential vCJD risk[a] (5th - 95th perc)[b]** | **Mean population –based potential vCJD risk[a] (5th - 95th perc)[b]** |
| ***Mean Total cumulative annual exposure and population risk*** | **1,800** | **243 million IU[c]** | 1 in 3,077 years (0-0)[d] | 1 in 35 years (0 - 1 in 8 ) |

[a] Mean population-based potential annual vCJD risk – the risk of potential vCJD infection for the entire population of 1,800 based on animal model dose-response information.
[b] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[c] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[d] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

## *Estimated annual potential vCJD risk associated with human pdFVIII used to treat severe von Willebrand disease (vWD)*

Individuals with vWD have varying severities of disease; those with Type 3 disease have the severest form of the disease. This executive summary specifically addresses potential vCJD exposure and risk for persons with severe vWD (Type 3) who are assumed to use larger amounts of pdFVIII product and thus, may be at higher risk. FDA estimates that approximately 250 vWD patients have severe vWD disease in the United States and use human pdFVIII products to control their disease (Table II.A.). Results from the risk assessment model for young vWD patients and adult vWD patients treated with pdFVIII product that is assumed to have a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in Table II.A. Generally results from the model are expressed for patients with vWD for two groups, either Prophylaxis or Episodic treatment. FDA obtained data on FVIII utilization from the Centers for Disease Control (CDC). The data were generated as part of a collaborative effort between CDC and six states; the study was conducted from 1993 –1998. Annual usage of product by vWD patients was estimated based on an assumption that this patient class largely uses Humate-P®. Totaling the model results for the lower prevalence estimate of ~1.8 per million reveals that the 250 severe vWD patients in the US (Table II.B.) are predicted to have an average potential vCJD infection risk for the population of 1 infection in 28,450 years. At the higher prevalence estimate, the average potential vCJD infection risk for this population is 1 infection in 405 years

**Table II.A. Model Results for von Willebrand Disease (vWD) Patients[a] with Severe Disease: Predicted Potential Annual vCJD Risk:**

- Assuming a reduction from manufacturing of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

| YOUNG vWD (≤ 15 yrs of age) | | | | |
|---|---|---|---|---|
| | | | 4 - 6 $\text{Log}_{10}$ Reduction | |
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| | **Est. Total Number patients in US** | **Mean quantity product used per person per year** | **Mean vCJD risk per person per year[b] (5th - 95th perc)[c]** | **Mean vCJD risk per person per year[b] (5th - 95th perc)[c]** |
| ***Prophylaxis*** | 39 | 165,713 IU[d] (9876, 454306) | 1 in 4.7 million (0-0)[e] | 1 in 52,000 (0 - 1 in 13,000) |
| ***Episodic*** | 60 | 11,045 IU[d] (1025, 34352) | 1 in 48 million (0-0)[e] | 1 in 971,000 (0 - 1 in 293,000) |
| ADULT vWD ( > 15 yrs of age) | | | | |

| | | | | |
|---|---|---|---|---|
| *Prophylaxis* | 73 | 186,880 IU[d] (16910, 539877) | 1 in 4.1 million (0-0)[e] | 1 in 46,300 (0 - 1 in 11,000) |
| *Episodic* | 78 | 86,923 IU[d] (2182, 240338) | 1 in 10 million (0-0)[e] | 1 in 1 million (0 - 1 in 24,000) |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.
[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.
[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[d] IU - represents International units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

**Table II.B. Von Willebrand Disease (vWD) patients[a] with Severe Disease: Predicted Total Population-based Potential vCJD Risk:**

- *Assuming a reduction from manufacturing of 4-6 log $_{10}$, and*
- *Two different UK vCJD prevalence estimates.*

| | | | 4 - 6 $Log_{10}$ Reduction | |
|---|---|---|---|---|
| | | | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani ( 2005) | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225*** *by Hilton, et al (2004)* |
| | **Est. Total Number severe vWD patients in US** | **Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c]** | **Mean[b] population –based Potential vCJD risk (5th - 95th perc)[c]** | **Mean[b] population –based Potential vCJD risk (5th - 95th perc)[c]** |
| ***Mean total annual exposure and population risk*** | 250 | 29.9 million IU[d] (3013, 311745) | 1 in 28,450 years (0 - 0)[e] | 1 in 405 years (0 - 1 in 76) |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or sodic treatment with Humate P only and no record of inhibitor.
[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.
[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[d] IU - represents International units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FVIII plasma pool would be rare and more than 90% of FVIII product lots (of vials) would not be predicted to contain vCJD agent.

## Conclusions

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Although donor deferral criteria in place

since 1999 have reduced the risk of donation by exposed persons, some are not deferred and potentially may donate plasma that contains the vCJD agent. However, the model suggests that the likelihood of a vCJD contaminated plasma pool is low.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the model predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from1 in 15,000 for the higher vCJD prevalence estimate and high product usage to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. Due to the wide range of methods used for currently available clearance studies, the results themselves, and gaps in information, it is not possible at this time to determine with any certainty if a specific product may be less or more safe than another.

Although results of the model suggest exposure to vCJD agent is possible, and there is a potential risk of infection that is likely to be very low, it is not possible for the model to provide a *precise* estimate of the vCJD risk in general, or of the actual risk to individual patients. Although the actual risk is highly uncertain, the risk assessment model indicates that the most important factors affecting risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, and the vCJD prevalence in the UK donor population.

*In considering the results of the risk assessment it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products, including pdFVIII. This includes patients receiving large amounts of plasma-derived products manufactured from UK plasma donations over a long period of time.* This observation also suggests that the actual risk of vCJD infection from pdFVIII is likely to be very low. The absence of cases does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

# RISK ASSESSMENT

## I. INTRODUCTION

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the UK in 1996 – and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the UK. Since December 2003, there have also been three reports in the United Kingdom (UK) of probable variant Creutzfeldt-Jakob disease (vCJD) transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness.

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the UK, where the risk is considered greatest, or elsewhere in the world. UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins.

Because only 3 cases of BSE (2 that originated in the US, 1 in Canada) have been reported in the US, the US vCJD risk from domestic beef is thought to be very low. However, some US residents (including blood and plasma donors) traveled to the UK, France and other countries in Europe since 1980 and may have been exposed to the BSE agent, and some of these donors may unknowingly be infected with vCJD. The UK had the largest epidemic of BSE among its cattle population and the largest human epidemic of vCJD, which as of August, 2006, reported 162 cases. The UK instituted strong food chain control measures to prevent the entry of high risk cattle tissues into its food supply in 1996; so risk after that time likely decreased considerably. France is considered to rank second in the world for risk for vCJD at this time, albeit at a much lower level than the UK, but higher than many other countries in Europe. As of August 2006 France has reported 20 cases of vCJD. Current US blood and plasma donation policies defer donors with a history of travel or residence to: the UK for a period of three months or longer (1980 – 1996); France, for a period of five years or longer (1980 - present); and other countries in Europe (blood donation only) for 5 years or longer (1980 – present). The CJD geographic donor deferral policy likely removes most of the vCJD risk; however, there may be residual risk in the US donor population for persons who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process.

FDA initiated a draft risk assessment of the potential vCJD risk for US manufactured pdFVIII in late 2004. A preliminary draft concept risk assessment model assessing the potential vCJD risks for US manufactured pdFVIII was presented at the February 8, 2005 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for review and comment. The committee largely agreed with the proposed approach. On October 31, 2005, FDA sought advice and

discussion on several risk assessment model inputs to be used in a risk assessment for US manufactured pdFVIII and potential vCJD risks. FDA has incorporated staff, peer reviewer comments, and technical advice provided by the TSEAC at the February 8, 2005 and October 31, 2005 meetings to develop this document "Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US".

This document quantitatively estimates the probability and level of exposure to the vCJD agent and the possible risk of vCJD infection in patients with severe hemophilia A (HA) and von Willebrand disease (vWD) patients with severe (Type 3) disease who have used human pdFVIII product manufactured in the US. Because BSE occurs at an extremely low level in US cattle (2 native born cows and 1 cow imported from Canada), the risk of plasma donors acquiring vCJD by consuming domestically produced beef is thought to be very low. Because of concerns about potential exposure to the BSE agent in US blood donors who traveled to or lived in the UK and other at risk European countries, FDA implemented donor deferral policies beginning in 1999. The policies are believed likely to reduce the possible risk from blood donors potentially exposed to BSE agent by ~ 90%. However, it is possible that a small number of non-deferred US donors may still have been exposed to the BSE agent during extended travel or residence in the UK, France or countries of Europe and may be at risk for vCJD. Some of these donors may have been unknowingly infected with vCJD through eating beef from BSE-infected cattle and then contributed donations to plasma pools used to manufacture pdFVIII in the US.

## Scope of the risk assessment

The scope of this FDA risk assessment evaluates the annual potential exposure to the vCJD agent and risk of vCJD infection through human plasma-derived Factor VIII (pdFVIII) product manufactured in the US. Risk for these products was estimated for the baseline year of 2002 but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. The FDA risk assessment specifically addresses pdFVIII used to treat patients with severe HA and severe vWD.

The FDA risk assessment utilizes a computer-based simulation model that evaluates successively the impact on vCJD risk of individual processes used in the production of human pdFVIII starting with plasma donation, extending through manufacturing steps, and finally, addressing utilization by various patient subpopulations. A few major elements of the model greatly influence vCJD risk. The most influential are manufacturing processes, which may reduce or eliminate the amount of vCJD agent in the final product. The amount of product used by patients in different clinical scenarios also has a significant impact on risk. Additionally, the prevalence of vCJD in the United Kingdom population, which is used to estimate vCJD prevalence in US donors who resided in or traveled to the UK and other countries of Europe, has a significant effect on the risk estimate.

The risk assessment model estimates the potential for vCJD exposure and the potential risk of vCJD infection for patients receiving pdFVIII from plasma collected in the US and the accompanying uncertainty of these estimates. Because scientific data on the level of exposure to vCJD agent and the likelihood of certain human health outcomes, such as infection and illness, are lacking, the estimates generated may not be accurate. As a result of these and other large uncertainties, it is not possible to

provide a precise estimate of the vCJD risk to patients potentially exposed to the agent through plasma-derived products.

## Background

*Hemophilia and Factor VIII*

Patients with HA have an inherited, recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie et al 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a protein in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Patients with vWD have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually necessitate treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

FVIII from human plasma is manufactured in a number of different ways. FVIII manufactured from human plasma is purified by fractionation of the protein from large plasma pools containing thousands of donations of plasma. Because thousands of donations are used to assemble the plasma pools used in the manufacturing of pdFVIII, there is a possibility that a donation from a vCJD infected individual may be present in a large plasma pool used to manufacture pdFVIII. In turn, that may lead to exposure of product recipients to the vCJD agent and a risk of infection. Relatively recent advances in pdFVIII production technology, such as [redacted] have likely reduced potential exposure to the vCJD agent. However, further evaluation is necessary to more precisely determine the levels of vCJD clearance afforded by the manufacturing processes for each human pdFVIII product.

There are two approaches for the clinical treatment and control of HA using pdFVIII: (1) episodic treatment and (2) prophylaxis. Episodic treatment involves the administration of FVIII in response to bleeding episodes resulting from trauma or during and after surgery. Prophylaxis treatment requires administration of clotting factor concentrates on a regularly scheduled basis necessary to maintain a minimal level of FVIII (common acceptable trough level is 2-5% of base line level) to prevent bleeding episodes. In view of the demonstrated benefits of prophylaxis, the Medical and Scientific Advisory Council (MASAC) recommends that prophylaxis starting at an early age be considered as an optimal therapy for individuals with severe HA (MASAC 2001). Prophylaxis treatment requires

higher doses of FVIII than episodic treatment (Linden, Kolakoski *et al* 2003; Globe, Curtis *et al* 2004) and thus presents a potentially higher risk of vCJD to the patients than episodic treatment when human pdFVIII is used. Also, some HA patients develop antibodies to FVIII, called inhibitors, that limit the effectiveness of FVIII used in treatment. Inhibitors can develop with the use of either recombinant FVIII or pdFVIII products. In some cases the development of inhibitors is treated with immune tolerance therapy in which large doses of one million or more units of pdFVIII may be administered. Because of the large doses of pdFVIII used, immune tolerance therapy can pose a potential risk for vCJD exposure if vCJD agent were present in the pdFVIII product. As a simplifying assumption in the model we assumed that in a given year a patient received either exclusively prophylaxis treatment or episodic treatment, but not both.

### Risk Assessment Framework

This risk assessment generally follows the four step paradigm described by the National Research Council (NRC, 1983) and consists of: (1) hazard identification, (2) hazard characterization, (3) exposure assessment, and (4) risk characterization. The hazard identification portion of the risk assessment provides an in-depth overview and analysis of all data and information sources to establish a causal relationship between the hazard and adverse effects on humans. The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the probability of adverse consequence(s) such as infection, illness, etc., expected at a given dose at the individual, subpopulation, or population level. Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. Risk Characterization integrates the information from the hazard identification, hazard characterization and exposure assessment sections to characterize the probability and consequences of risk for individuals and populations.

## II. HAZARD IDENTIFICATION

The hazard identification portion of the risk assessment provides an in-depth overview and analysis of information from laboratory studies, epidemiological studies, the scientific literature, government reports and other credible or peer-reviewed sources of data that establish a causal relationship between the hazard and adverse effects on humans. In this risk assessment, the vCJD agent is the hazard, and potential exposure can occur in individuals who use plasma-derived products that may have been manufactured from plasma that may have contained a donation(s) from a vCJD-infected individual. To date, there is no epidemiological evidence suggesting that vCJD has been transmitted by use of plasma derivative products. However, the probable transmission of vCJD to three recipients of red blood cell products donated by donors later diagnosed with vCJD in the UK raises the possibility of vCJD transmission via plasma-derived products.

Human vCJD was first reported in the United Kingdom in 1996 (Will *et al* 1996). As of August 2006 over 195 cases, 162 of them in the UK, have been reported worldwide. Both vCJD and BSE belong to a class of fatal neurodegenerative diseases known as transmissible spongiform encephalopathies (TSEs). There is strong evidence and general agreement that vCJD results from infection of humans

most probably via dietary exposure to bovine spongiform encephalopathy (BSE)-contaminated beef (Knight 2004). The leading theory is that the transmissible infectious agent is a prion, or proteinaceous infectious agent, that is an altered but pathogenic form of the PrP protein that is normally present in cells. The altered PrP, herein referred to as $PrP^{TSE}$, consistent with terminology recommended by the World Health Organization, is highly stable and resistant to degradation by high heat and chemical treatments commonly used to denature infectious agents in the manufacture of plasma derivatives. The incubation period for TSEs is long. The mean incubation period of BSE in cattle is approximately 4.5 years. In humans, vCJD acquired through dietary exposure is thought to incubate approximately 10 –12 years or longer, and individuals become symptomatic only in the last few months of the disease, making early detection very difficult. Confirmation of vCJD requires postmortem examination of brain tissue to confirm diagnosis, but prion protein has been detected in tonsil and appendix tissue of asymptomatic individuals as long as two years prior to the onset of symptoms. There are currently no validated tests available to detect the disease in its early stages of infection or to detect the presence of TSE agents in blood.

**Transmission of TSEs through transfusion of blood products in animal models**

Transmission of different TSE agents through the transfusion of blood or blood products has been demonstrated in animal models on multiple occasions. At least four studies reported transmission via blood transfusion in the same animal species: sheep experimentally infected with BSE (Houston *et al* 2000), sheep naturally infected with scrapie (Hunter *et al* 2002), hamsters with scrapie (Rohwer 2004), and mice with a human TSE (Brown *et al* 1999).

Brown, Rohwer, Taylor (Taylor *et al* 2000) and others have attempted to estimate the amounts of intracerebral (i.c.) infectivity present in blood, which generally fell between 2 and 20 i.c. $ID_{50}$/ml. A recent study of scrapie-infected hamsters concluded that approximately 58% of the infectivity present in whole blood was associated with plasma (Gregori *et al* 2004). The model uses this more conservative estimate in the published literature and assumes that 58% of infectivity is associated with plasma.

**Transfusion transmission of vCJD in the United Kingdom**

In December 2003 the UK government announced that vCJD had likely been transmitted to a 69 year-old patient via blood transfusion. The patient had received non-leukoreduced red blood cells in 1996 from a donor who died three years later of vCJD. This first report was followed by the announcement in July 2004 of another probable case of transfusion-transmitted vCJD. The patient died of a ruptured aortic aneurysm without clinical evidence of vCJD, but postmortem testing detected $PrP^{TSE}$ in spleen tissue and cervical lymph node. In February 2006 a third case of probable transfusion transmitted vCJD was reported in the UK in a 31 year-old male; the patient had received a transfusion eight years earlier from a donor who died of vCJD 20 months after donation. None of the donors were known to have had vCJD at the time of donation.

It is possible that dietary exposure may have been responsible for some or all of the three cases that were reported after red blood cell transfusions; however, the probabilities for occurrence of either a single, or, particularly, two or three such events are small. As Llewelyn *et al* (2004) pointed out in their publication discussing the first presumed transfusion-transmitted case "the age of the patient

was well beyond that of most vCJD cases, and the chance of observing a case of vCJD in a recipient in the absence of transfusion transmitted infection is about 1 in 15,000 to 1 in 30,000." The combined probability that the first two transfusion cases, identified in two elderly patients in a small cohort of transfusion recipients—in an age group underrepresented among vCJD cases—both acquired infection from food is remote.

The presumptive transmission of vCJD via red blood cell transfusion in the UK raises the possibility that plasma derivatives may pose a risk. The UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These plasma derivative products included coagulation factors, as well as antithrombin III, and intravenous immune globulins. The derivatives of concern were manufactured from plasma of UK donors between 1980 and late in 1999, when--consistent with a decision announced in 1998—UK manufacturers stopped using UK plasma. The last expiry date for any of the UK products was in 2001. To date, no cases of vCJD have been reported in any recipient of plasma derivatives, either in the UK, where the risk is considered greatest, or elsewhere, including in patients who have received human plasma-derived coagulation products from implicated lots (e.g., lots manufactured from a pool containing plasma from a donor subsequently diagnosed with vCJD) made in the UK.

**The vCJD risk for travelers with history of extended travel or residence in the UK, France, and other countries in Europe and reduction of risk via donor deferral**

Public health control measures, such as surveillance, culling of sick animals, or banning specified risk materials, and others have been instituted relatively recently in many European countries, particularly in those with indigenous cases of confirmed BSE, in order to prevent potentially BSE-infected tissues from entering the human food supply. Since 1996, the UK has instituted some of the most stringent of these control measures, including a program that excludes all animals >30 months of age and prevents high risk tissue from slaughtered animals from entering the human food and animal feed supplies. In June 2000, the European Union Commission on Food Safety and Animal Welfare strengthened the European Union's BSE control measures by requiring all member states to remove specified risk materials from animal feed and human food chains; as of October 1, 2000 such bans had already been instituted in most member states.

Travelers to and residents of the UK, France and other countries in Europe during the period of BSE pandemic concern are possibly at increased risk of vCJD. However, the risk can not be determined precisely due to factors such as the great uncertainty about incubation period of the disease, the sensitivities of each country's surveillance for BSE and vCJD, the compliance with and effectiveness of public health measures instituted in each country to prevent BSE contamination of human food, and the cattle products from one country that are distributed and consumed elsewhere.

In the UK, the current risk of acquiring vCJD from eating beef and beef products appears to be extremely small, perhaps about 1 case per 10 billion servings (CDC, 2005). In the other countries of the world, this current risk, if it exists at all, would not likely be any higher than that in the UK if BSE-related. The implementation of animal and public health control measures has caused the prevalence of BSE to decline. The US blood donor deferral criteria currently in effect focus on the

time (cumulatively 3 months or more) that a person lived in the UK from 1980 through 1996, whereas for the rest of Europe the criteria focus on the time (cumulatively 5 years or more) that a person lived in these countries from 1980 through the present. This deferral policy is expected to reduce the risk of vCJD transmission via blood and plasma donations from potential infected donors.

**Two cases of vCJD in US residents with history of residence in the UK**

In 2002, the first case of vCJD was reported in the United States in a 22-year-old woman living in Florida. She is believed to have become infected with vCJD during her residence in the UK. The patient was born in Great Britain in 1979 and immigrated to the United States in 1992. In early November 2001, the patient was evaluated for depression and memory loss. In late January 2002, the patient was transported to the United Kingdom where her condition worsened. The diagnosis of vCJD was confirmed by western blot and immunohistochemical analysis. The patient died in June 2004, approximately 32 months after illness onset (Belay *et al* 2005).

A second case of vCJD was diagnosed by the UK National Creutzfeldt-Jakob Disease Surveillance Unit in November 2005 in a 30-year old man who resided in Texas during the period 2001-2005 (CDC 2006). The onset of symptoms occurred in early 2005 while the man was in Texas. He returned to the UK and died of the disease in early 2006. A postmortem examination confirmed the diagnosis of vCJD.

**Surveillance studies to detect CJD and vCJD in patients with hemophilia**

**Studies in the United States**
Because of the large number of blood products used, persons with hemophilia might be expected to be at risk of developing transfusion-related vCJD or classical Creutzfeldt-Jakob disease (CJD). However, a study conducted by the US Centers for Disease Control (CDC) (Evatt *et al* 1998) examined the brains of 24 decedents with a history of bleeding disorders and dementia and found no evidence of CJD in any of the cases.

Another study conducted by the CDC and the Hemophilia Treatment Center identified no cases of clinical diagnosis of CJD among over 12,000 HA patients who have been assessed since 1996. (Evatt *et al* 1998)

**Studies in the United Kingdom**
A study in the UK (reference: Lee *et al* 1998) conducted post mortem histological examination of the brains of 33 hemophilia patients who were treated with coagulant factor concentrates spanning the years from 1962 – 1995 and observed no evidence of vCJD.

In summary, the experimental and epidemiological evidence indicates the risk of blood transmission of vCJD is no longer theoretical but a real possibility. To date, there is no evidence suggesting that either human CJD or vCJD have been transmitted by use of plasma derivative products. However, transmission of vCJD via red blood cell tranfusions has likely occurred (Llewelyn *et al* 2004). Because the vCJD agent may be associated with plasma, it is plausible that plasma derivatives potentially pose a risk of transmitting the disease.

# III. HAZARD CHARACTERIZATION

The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the adverse consequence(s) such as infection, illness, etc., at the individual, subpopulation, or population level. Determining dose-response relationships can be difficult to accomplish because data are limited, especially exposure and outcome data for humans. Other factors such as characteristics of the hazard (e.g. strain, chemical make-up, etc.), route of introduction, genetics of exposed individuals, influence the dose-response relationship but are often difficult to characterize. Often in lieu of human data, animal data are used and appropriately extrapolated as best as is possible to estimate the dose-response relationship for humans.

Another challenge is estimating the probability of infection when the exposure to TSEs is small and/or occurs repeatedly over a period of time. It is unknown whether for TSE diseases there is a minimal amount of the agent (presumably the prion protein $PrP^{TSE}$) or threshold that is needed to initiate infection in an individual. This phenomenon is seen with many other pathogens such as viruses or bacteria, for which infection requires exposure to at least one, and often more, units of the infectious agent. Furthermore, it is not known whether the effects of small multiple exposures over a period of time are cumulative and may result in the possibility of infection and disease equivalent to a single, larger exposure (e.g., via intracerebral injection in laboratory animals). Some risk assessments have made assumptions concerning the exposure and dose for TSE agent that leads to infection. For instance, the Det Norske Veritas (Feb 2003) blood products risk assessment assumes that exposure to infectivity, quantified in $ID_{50}$ units, is cumulative over the period of one year. Based on advice from the TSEAC (2005), and consistent with suggestive data from studies of TSE agents in animal models (Diringer *et al* 1998, Jacquemot, *et al* 2005), FDA also assumes that exposure to vCJD $ID_{50}$ is cumulative over a one year period. The $ID_{50}$ is the common metric used to quantify the infectivity of TSEs. One $ID_{50}$ is defined as the amount of infectious material or tissue that is necessary to initiate infection in 50% of the treated population. The route of exposure to TSE infectious material influences the efficiency of transmission of the disease. Based on advice provided to FDA by the TSEAC (October 31, 2005) the model assumes that transmission via the intravenous (i.v.) route is between 1 and 10 times less efficient than the transmission via the intracranial (i.c.) route.

In estimating the dose-response relationship for TSEs one could use a strict interpretation of the $ID_{50}$ and assume a linear relationship between exposure and infection. In the pdFVIII model FDA assumed there was a linear relationship between the exposure dose of vCJD agent and the probability of infection. The $ID_{50}$ relationship used in the model was based on infectious TSE units estimated from rodent model studies (Brown 1998, 1999; Rowher 2004). We further assumed there was no threshold or minimum dose necessary to initiate infection, that is, exposure to even low quantities of vCJD agent has a probability of initiating infection in an individual, albeit the probability of infection would likely be low at low levels of exposure. The model further assumes that in such a case exposure to 1 $ID_{50}$ would suggest a 50% probability of infection, exposure to 0.1 $ID_{50}$ would suggest a 5% probability of infection, and so on. However, given the lack of information and high degree of uncertainty on the dose-response relationship because of the limited data available for TSE agents, it is plausible that low level exposures, even on a chronic basis, may not attain a threshold or minimum quantity of agent necessary to initiate infection in humans. Again, FDA makes a conservative

assumption that low-level exposure(s) over the period of one year to any quantity of vCJD agent could potentially lead to infection and that there is not a minimum dose necessary to initiate infection.

There are considerable uncertainties in determining the correct form for the vCJD-human dose-response model. For instance, the nature of the dose-response line, its slope, or whether it is more accurately described using a dose-response curve is uncertain because animal data are so limited and human data are not available. The FDA risk assessment estimates the potential individual risk of infection and assumes that a linear interpretation of the rodent model accurately reflects the pathology and progression of vCJD infection and disease in humans, but it may not. Furthermore, exposure to the vCJD agent may not necessarily lead to infection, and vCJD infection may not necessarily produce symptomatic vCJD disease or illness in an individual or population.

# IV. EXPOSURE ASSESSMENT

Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount (dose) of a hazardous agent to which a person or population may be exposed. This exposure assessment specifically addresses the probability of exposure and, if present, the quantity of vCJD agent that may potentially be present in plasma-derived FVIII products manufactured in the United States. The administration of pdFVIII and, thus, the route of exposure, is intravenous.

Plasma pools consisting of 6,000 or more donations collected from US plasma donors are used as the starting material from which a number of plasma-derived products are purified, including pdFVIII, which is addressed in this assessment. Because of the relatively large number of donations per plasma pool, there is a small probability that even in the United States some of the pools may contain a donation from a donor who may unknowingly be infected with vCJD, but who does not meet criteria for donor deferral, or who meets those criteria but fails to be deferred due to the limitations of the screening process.

**Overview of Model for pdFVIII**

**Module 1 – Estimation of the prevalence of vCJD in the UK.** Variant CJD prevalence in the UK was used in our model as the basis for estimating vCJD prevalence in US plasma donors. The model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and may have had dietary exposure to the BSE agent during their stay.

Two different data sources were used to estimate UK vCJD prevalence.

- An epidemiological modeling-based approach estimates a UK vCJD case prevalence of approximately ~1.8 cases per million population (Clarke and Ghani 2005).
- A tissue surveillance-based estimate for UK vCJD infection prevalence was generated using data from Hilton *et al* (2004) and yielded a mean estimate of 1 case per 4,225 – but was further adjusted to account for age of patients surveyed.

**Module 2 –vCJD Prevalence in US Plasma Donors and Pools.** This module estimates the number of US plasma donors that may potentially be infected with vCJD and the percentage/number of pools containing donations with vCJD agent. This module uses survey data to determine US plasma donors potentially at risk for vCJD, including those with a history of:

- Dietary exposure to BSE-contaminated beef during long term travel or residence in the UK (1980-1996), France and other countries in Europe (since 1980),
- Military service - posted on or residing near military facilities in Europe; and
- Transfusion with blood collected in Europe, or Euroblood.

US plasma donors potentially at risk for vCJD were further characterized by:

- Country of travel or residence,
- Specific duration of travel or residence, year of travel or residence,
- Age of donor, rate and frequency of plasma donation,
- Number of donations per pool, and type of plasma pool (source or recovered), and
- Effectiveness of donor deferral policies.

**Module 3 - pdFVIII Manufacturing and Processing.** This portion of the model calculated the likelihood and number of plasma pools potentially containing vCJD agent and the quantity of agent per plasma pool and pdFVIII vial based on:

- The probability of and predicted quantity of infectivity (i.v. $ID_{50}$) present per donation and pool
- Reduction in the quantity of potential vCJD agent during manufacture, and
- Total yield or quantity of pdFVIII produced from the plasma pool.

**Module 4 - Utilization of pdFVIII by Hemophilia A patients.** The potential exposure of an individual HA patient to the vCJD agent through use of pdFVIII was estimated in the model based on:

- the total quantity of pdFVIII used per year, and
- the estimated potential quantity of vCJD agent predicted in the pdFVIII product.

The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen and was estimated using data from a Centers for Disease Control (CDC) sponsored study by 6 states by HA patients from 1993-1998

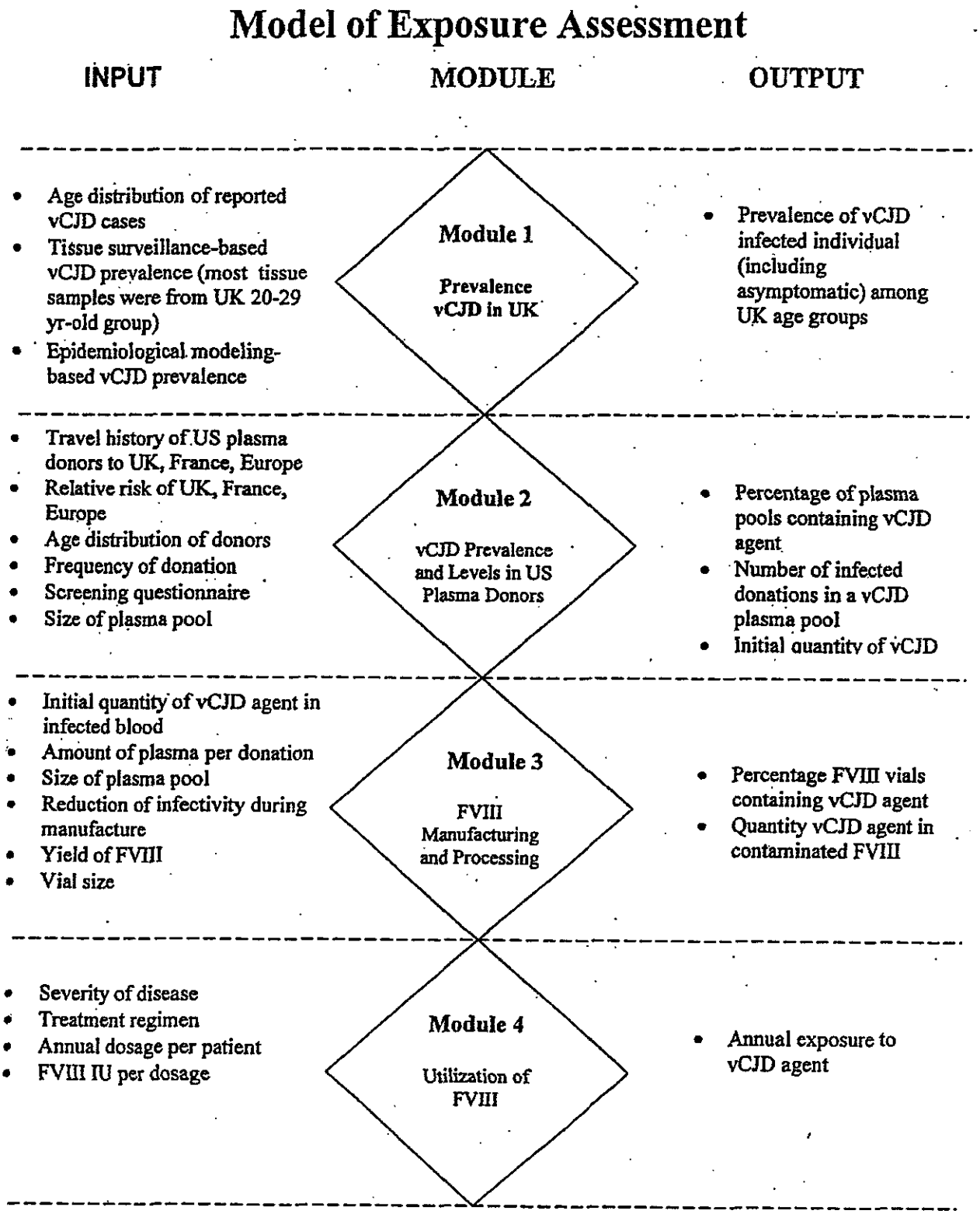
This risk assessment provides outputs that estimate annual exposure for several patient subpopulations with

Severe HA disease for persons in the following clinical treatment groups:

- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

vWD for adult (≥15 yrs of age) and young (≤15 yrs of age) persons, including those in either clinical treatment group: Prophylaxis or Episodic.

Figure 1 Exposure assessment diagram



## IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

The potential prevalence of vCJD in the UK was and continues to be dynamic and changes throughout time as people are exposed to the BSE agent, infected with vCJD, develop the disease and eventually die. Variant CJD exposure and infections in the UK population likely occurred in proportion to the UK BSE epidemic which peaked in 1992. The first human vCJD cases were referred to UK public health authorities in 1994. To date, the number of cases per year in the UK reached a maximum of 28 in the year 2000, and since then has been declining annually with a total of 5 deaths in year 2005.

The FDA model assumes that the major source of potential vCJD in the US would likely be associated with plasma donors with a history of travel and residency in the UK, France or other countries in Europe since 1980 and who may have had dietary exposure to the BSE agent during their stay. The potential vCJD prevalence in US plasma donors with a history of travel to BSE countries since 1980 was estimated based on the UK vCJD prevalence. For US donors the UK vCJD prevalence was adjusted based on the proportion of time spent in the UK, the year of travel and age of the donor. Calculation of the potential vCJD risk for donors who traveled to France was estimated relative to the UK risk (or the relative risk), based on the amount of UK beef imports, the number of domestically acquired vCJD cases, and other factors. The relative risk for vCJD for France was assumed to be 0.05 times that of the UK risk (or 0.05 times the UK vCJD prevalence). Applying similar criteria for other countries in Europe their relative risk was assumed to be 0.015 times that of the UK. Risk was calculated in the model for donors by multiplying the UK vCJD prevalence by either 0.05 for travel to France or 0.015 for travel to other European countries and further adjusting the prevalence to account for factors such as the proportion of time spent, the year of travel and age of the donor.

The prevalence of vCJD in the UK is difficult to estimate because of the long incubation period of the disease and a lack of a validated test that can detect infection in its asymptomatic stages. The prevalence of asymptomatic vCJD infections in the UK was estimated in the FDA model using two different approaches based on two different data sources:

- **An epidemiological modeling-based approach that combined information from a study by Clarke and Ghani (2005) and diagnosed vCJD cases for 2002 and 2003 was used to estimat a UK vCJD case prevalence of approximately ~1.8 cases per million population.** There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. Several factors used in epidemiologic modeling approaches are difficult to quantify and add uncertainty to the final estimated number of future vCJD cases. These factors include: the intensity of human exposure to the BSE agent, incubation period, time of infection, and whether illness will develop in individuals who are not homozygous for methionine at codon 129 of PrP. All cases of vCJD to date have occurred in individuals who are homozygous for methionine at this location. A more detailed description of the derivation of the epidemiological modeling-based estimate and further discussion of the limitations of the approach can be found in section IV. A. 1. below.

- **A tissue surveillance-based estimate for UK vCJD prevalence was generated using the results of a UK study that tested stored tonsil and appendix tissues collected from patients in the 1990s for the accumulation of prion agent (Hilton *et al* 2004).** The study yielded a much higher estimate of 1 in 4,225 (237 infections per million). However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be

considered. A total of 3 positive appendix tissues were identified among 12,676 tissue samples tested, yielding a mean UK vCJD prevalence estimate of 1 case per 4,225. This prevalence estimate was further adjusted to account for the age of the patients surveyed (mostly 20 – 29 year olds) to arrive at a total population-based estimate of UK vCJD prevalence.
This study was not controlled using tissues from a non-BSE exposed population, and false positive interpretations of the findings cannot be ruled out. It is also not known whether this staining of appendiceal tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be considered. A more detailed description of the derivation of the tissue surveillance-based estimate and further discussion of the limitations of the tissue surveillance study can be found in section IV. A. 2. below.

Two spreadsheet models were developed for the FDA risk assessment – one for each of the two prevalence estimates – but otherwise the models were identical in all other ways. We describe the surveillance variables and assumptions in the sections immediately below.

## IV. A. 1. UK vCJD prevalence estimated using epidemiological modeling results (Clarke and Ghani 2005) and diagnosed vCJD cases for 2002 and 2003

The first approach used to estimate UK vCJD prevalence in the FDA model relied largely on epidemiological modeling results (Clarke and Ghani 2005) that estimated future 70 vCJD cases in the UK for the years 2004 – 2080. Since the FDA model estimates the baseline vCJD infection risk for pdFVIII product used in the year 2002, we assumed the potential risk for US donors should be calculated based on a UK vCJD prevalence that included all vCJD cases and potentially incubating vCJD infections in the year 2002. Therefore to estimate the number of cases and future vCJD infections in the UK for the years 2002 – 2080 we added the 32 known diagnosed cases in years 2002 and 2003 and the estimated future 70 vCJD cases (Clarke and Ghani 2005). We assumed that the 70 future cases predicted by Clarke and Ghani (2005) would be incubating vCJD infection in 2002. Therefore, the FDA model estimated an average of 102 cases and incubating vCJD infections for the year 2002 and assumed a 95% confidence interval of 42 – 222 cases. The results of the input information and calculations for the number of vCJD cases in the UK in 2002 are summarized in **Table 4.1.** Assuming the population of the UK in 1997 is approximately 58 million, the prevalence of vCJD (United Kingdom Office for National Statistics, 1997) would be a mean of approximately 1.8 vCJD infections per million population (102 potential vCJD cases / 58 million).

**Table 4.1. FDA model estimation of UK vCJD cases for years 2002 – 2080.**

| | **Diagnosed vCJD cases in the UK** (Health Protection Agency, 2006) | | | **Estimation of future UK vCJD cases** (Clark and Ghani 2005) | **FDA model: Estimation of UK vCJD cases for years 2002 - 2080** |
|---|---|---|---|---|---|
| **Year(s)** | **2002** | **2003** | **Total** | **2004 - 2080** | **2002 - 2080** |
| **Number of vCJD** | 16 | 16 | 32 | 70 (10 – 190) | 102 (42 – 222) |

| cases | | | | | |
|---|---|---|---|---|---|

There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. Many of the published models of future vCJD cases or vCJD incidence in the UK, including Clarke and Ghani (2005) and Cooper and Bird (2003), use simplifying assumptions in generating their predictions. Although these simplifying assumptions are a necessary part of vCJD case estimation efforts, they contribute considerable uncertainty to the final case estimates. Generally, the types of assumptions used to estimate vCJD cases fall into four general areas. First, the models must estimate the number of clinical and pre-clinical BSE-infected cattle slaughtered in the UK to estimate the intensity of human exposure to the BSE agent. Second, they assume a level of effectiveness of the 1989 Specified Ban on Offals which was assumed to reduce the quantity of infectious BSE agent in the food supply, thereby reducing human exposure in the UK. Third, the models generate an appropriate mathematical representation (or statistical distribution) for the incubation period, which is represented by many usi a unimodal statistical distribution. There may be constraints on the incubation period used in the model (e.g., the vCJD incubation period of all individuals in the population would not exceed 40 years, etc.). Fourth, many of the modeling approaches incorporate age-specific dependencies that influence exposure, susceptibility to the disease, and incubation period. Depending on the assumptions used, estimates of future cases of vCJD have varied considerably. Past estimates of vCJD cases from epidemiological models predicted from 250 to 440 future cases under certain assumptions (d'Aignaux et al 2001). As actual reported vCJD cases peaked in 2000 and have since been declining, predicted estimates of future cases have decreased (Boelle et al 2003, Clarke and Ghani 2005, Cooper and Bird, 2003).

There are additional uncertainties in predicting future vCJD cases that might arise from individuals with different genetic backgrounds and susceptibilities in the UK population. To date, all known cases of vCJD have occurred in individuals that were methionine homozygous (MM genotype) at codon 129 of the prion protein gene (PNRP). Recent research has identified two individuals who were valine homozygous (VV genotype, also called non-MM genotype) at PRNP codon 129 (Ironside *et al* 2006) among the three prion protein positive samples identified by Hilton *et al* (2004). Clarke and Ghani (2005) did incorporate the possibility of wider genetic susceptibilities in some of their estimates of future vCJD cases. However, because no cases of clinical vCJD have been identified in individuals with non-MM genotype, it is uncertain whether these individuals will in fact develop or transmit clinical disease. Therefore, any estimation of the incubation period for potential cases with the non-MM genotype would rely heavily on assumptions, which adds considerable uncertainty to any estimate of the size or number of cases in a possible secondary wave of vCJD cases that might occur in non-MM individuals.

## IV. A. 2. UK vCJD Prevalence derived from a Tissue Surveillance study

We used a second approach for estimating UK vCJD prevalence drawing on results from a tissue surveillance study that tested lymphoreticular tissue samples (tonsils and appendices) for prion protein accumulation. The study was a retrospective survey of stored tonsil and appendix tissues surgically removed from UK patients in 1995 and subsequent years. The authors identified appendix samples from 3 patients as positive for lymphoreticular accumulation of prion protein out of a total of 12,674 patient samples tested (Hilton *et al* 2004). No tonsil biopsies showed such findings. The significance of the detection of prion protein in the appendix is not certain, and it is not known whether this test is a

reliable marker for either vCJD pre-clinical infection or the ultimate development of disease. Nor is it known whether or not such detection is a marker for an individual's potential capability to transmit infection through blood donation. Results from the tissue surveillance study are summarized in **Table 4.2.** Assuming the sensitivity and specificity of the testing method is 100%, this translates roughly to a vCJD prevalence of of 237 cases per million (95% CI: 49 – 692 cases per million) for all age groups. The authors (Hilton et al 2005) indicated that approximately 60% of the samples tested (from 7,600 patients) came from patients 20-29 years of age. Among the 20-29 year old group we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million.

**Table 4.2. Summary of surveillance testing of tonsil and appendix tissues in the UK.**

| Reference | Ages of population examined | Years tissue taken | Number of positives | Total samples examined | Rate per million (95% CI) |
|---|---|---|---|---|---|
| Hilton DA, et al. 2004 | 10 – 60+ yrs (60% of patients were 20-29 yrs) | 1995 - 1999 | 3 Appendices | 14,964 Appendices 1,739 Tonsils 4,029 excluded | 237/million (49–692 per million) |

There are some possible limitations of using the Hilton *et al* tissue surveillance study in estimating vCJD prevalence. In their tissue survey, Hilton *et al* stressed that there were uncertainties and suggested caution in attempting a prevalence estimate for infection or a prediction of future vCJD cases in the UK based on detection by immunohistochemical staining of lymphoreticular accumulation of prion protein in three of 12,674 adequate tissue samples studied. First, because the stage of vCJD infection during which the appendix first accumulates detectable amounts of abnormal prion protein is not known and because the accumulations might not be uniformly distributed throughout the tissue, the prevalence of infection might have been underestimated. Second, because the study design (lacking examination of a large number of similarly obtained appendices from a non-BSE-epidemic country) did not permit an estimate of specificity of the method or an independent confirmation of results, it is possible that the results might have been false positives leading to an overestimation of prevalence. In their paper the authors stated: "Although immunohistochemical accumulation of PrP in lymphoreticular tissues has not been demonstrated in any disease other than vCJD, the significance of the positive samples in this study is not certain. In one case, the immunohistochemical pattern of immunoreactivity resembled that seen in appendix tissue from pre-clinical and autopsied cases of vCJD, but in the other two cases, a more finely granular pattern of staining was present in relation to follicular dendritic cells, raising the possibility that these may be false positives. However, we have been unable to demonstrate PrP immunoreactivity in a range of other disorders including other human prion diseases, neoplastic disease, or a range of inflammatory conditions."

**Assumption used in the model:** All vCJD cases that occur after 2002 are incubating in year 2002.

Prevalence of vCJD among the UK population and the vCJD risk from using plasma-derived factor products are expected to be different from year to year since 1980. In this risk assessment, the potential vCJD risk for pdFVIII products was estimated for the baseline year of 2002, but the results and conclusions also are likely to reflect the current vCJD risk for recipients of pdFVIII. Prevalence of vCJD in 2002 for a specific age population in the UK was extrapolated from two estimates of prevalence discussed above based on age information of reported vCJD cases. The prevalence derived from above two different approaches varied by approximately 130 fold. The discrepancy reflects the limitation on the current knowledge of the disease. In order to evaluate the impact of uncertainty in estimation of vCJD prevalence, the FDA risk assessment provides estimated risk outcomes stratified by two estimates of prevalence.

**Assumption used in the model:** Our model assumed that distribution of asymptomatic cases across age groups would be the same as the distribution of observed symptomatic cases.

Additional technical information and details of analyses and modeling approaches are provided in Appendix A under section A - IV.A.

## IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2)

The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model.

This module estimates the annual number of plasma pools that are used to manufacture pdFVIII from plasma collected in the United States, the number of pools that potentially contain a donation from an infected plasma donor, and the potential quantity of vCJD agent that may be present in a positive pool. The potential vCJD risk for US plasma donors is likely associated with dietary exposure to BSE agent during periods of travel or residence in the at-risk geographic areas where BSE occurred. The percentages of blood donors with a history of travel or residency in BSE countries, who are military members who resided in bases in UK and elsewhere in Europe during 1980-1996, and who are recipients of "Euroblood" were obtained from 1980-1996 Blood Donor Travel Survey conducted by American Red Cross (TSEAC 2000). The percentage was calculated by destination (e.g., the UK, France or other European countries) and duration of travel.

Two different types of plasma are used in manufacture of pdFVIII. Source Plasma is collected through plasmapheresis, a process that separates red blood cells from plasma and returns red blood cells to the donor. Recovered plasma is prepared from whole blood units collected from blood donors. Source

Plasma accounts for approximately 80% of the total plasma collected annually in the United States, and recovered plasma accounts for the remaining 20%. Source Plasma donors are usually younger than blood (recovered plasma) donors, and are thought to travel less so presumably their vCJD risk may be somewhat lower than that of blood donors. However, because of their younger age demographic, Source Plasma donors are likely to be more susceptible to vCJD infection. Additionally, Source Plasma pools are usually smaller and contain larger volume donations (an average of 700 milliliters) from fewer donors than recovered plasma pools (average volume of a donation is ~200 milliliters). Plasma from fewer donors reduces the chance that a plasma pool may contain a donation from an infected donor. However, because Source Plasma donors are allowed to donate more frequently, and give more plasma per donation, there is a greater chance that if a vCJD infected donor were in the Source Plasma donor pool that they may contribute multiple donations to a single plasma pool or donate to multiple pools. However, blood deferral policies instituted beginning in 1999 are believed to have reduced the risk of vCJD donations by more than 90%. The effectiveness of the deferral policy in removing potential vCJD risk from the donor and donation pool is included in the FDA model. Because of the unique characteristics and potential differences in risk for Source and recovered plasma donations and plasma pools, the FDA risk assessment modeled Source and recovered plasma pools separately, and considered factors that may result in different risk for pdFVIII product made from each of the two types of plasma.

## IV. B. 1. a. Annual US plasma donors and characterization by age

Age is an important factor in estimating potential vCJD risk for US plasma donors. The FDA model is organized by age groups 18 and 19 yr olds, 10-14, 15-29, etc. (by five yr age groups to age 69) and calculates all risk information and makes all adjustments based on age groups. Each of these age groups forms a "bin" and in each of these bins donors are categorized by country of travel, vCJD prevalence (or relative risk) for country of travel, duration of travel, year of travel, type of donation (Source or recovered), donation rate, etc. The output at the end of this portion of the model is an estimation of the number of US donors in each age group that are potentially infected with vCJD. The model further incorporates the effect of FDA donor deferral policies, implemented beginning in 1999, that are believed likely to reduce the possible risk from blood donors potentially infected with vCJD by ~ 90%.

This specific portion of the model estimates potential age specific vCJD risk for both Source and recovered plasma donors. As mentioned above, donor age is an important factor associated with frequency of travel and susceptibility to the disease and influences the vCJD risk for a particular type of donor. For instance, Source Plasma donors as a group are generally younger than recovered plasma donors (see percentages of donors for Source and recovered plasma donors by age group in **Table 4.3**). Also, the younger Source Plasma donor population likely travels less, and thus, likely has a lower potential vCJD risk. However, this lower risk may be offset by the possibility that younger persons may be more susceptible to infection by the vCJD agent. The purpose of this portion of the model is to characterize plasma donors and their donations according to donor age to more accurately estimate the number of potential vCJD infected donors and donations containing vCJD agent. In turn this information will be used to estimate the probability that a plasma pool used in the manufacture of pdFVIII may contain a donation with vCJD agent.

Additional technical information and details of analyses and modeling approaches are provided in Appendix A under section **A - IV.B.**

**Table 4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups**

| Age group | <10 | 10-14 | 15-19 | 18-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | >70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Reported vCJD cases in UK (through 2003)[a] (%) | 0 | 5 (3.4%) | 27 (18.4%) | | 32 (21.8%) | 30 (20.4%) | 22 (14.9%) | 13 (8.8%) | 5 (3.4%) | 3 (2%) | 5 (3.4%) | 0 (0%) | 5 (3.4%) | | |
| Age distribution of US Source Plasma donors (%)[b] | 0 | 0 | 0 | 12% | 29.3% | 14.1% | 14.1% | 9.6% | 9.6% | 5.8% | 5.8% | 0% | 0% | 0% | 0% |
| Age distribution of US Blood (Recovered plasma) donors[c] | 0 | 0 | 0 | 5% | 13% | 8% | 10% | 12% | 13% | 12% | 11% | 7% | 4% | 5% | 0% |

[a] Hilton *et al.* 2004

[b] Plasma Protein Therapeutics Association (Jan 07, 2005). Where data were organized in broader age group we allocated donor equally among smaller 5 year age groups

[c] Data provided to FDA by Westat in 2002

## IV. C. Estimation of the probability that a plasma pool may contain a donation from an infected donor that contains vCJD agent

The purpose of this section of the model is to estimate the prevalence of vCJD in US donors who may have been exposed to the BSE agent and potentially infected with vCJD during travel, residence, military service in the UK, France or other countries in Europe since 1980. The vCJD prevalence in U donors is then used to estimate the probability that a plasma pool may contain a donation from a vCJDinfected donor with infectious agent in their blood at the time of donation.

The starting material for manufacturing pdFVIII is a plasma pool containing donations from thousands of donors. The probability that a plasma pool contains a donation with vCJD agent is a function of the prevalence of vCJD in the US donor population and the total number of donors and donations present in a pool used to manufacture pdFVIII. For US donors with a history of travel, a key factor in estimating the vCJD risk is the region or country of travel, i.e., the UK, France or other countries in Europe, since 1980. From there we make several adjustments to the vCJD prevalence for US donors. The model incorporates the age of donors into the estimate of vCJD prevalence – donation rate for various age groups is important since the majority of donors are less than 40 years of age. Furthermore, vCJD prevalence for each age group is determined. Since vCJD primarily affects younger persons (median age 28 yrs) and donors are younger – they are at particular risk for vCJD and may unknowingly transmit the agent via donations. The model further adjusts vCJD prevalence based on the year of travel – for instance, a traveler in 1992 that visited the UK at the height of the BSE epidemic faces a higher BSE exposure risk and risk of vCJD infection than someone who traveled to the UK in 1997 after more

stringent food controls were implemented in the UK. Also, the model incorporates information on the duration of the travel based on survey information of travel history for whole blood users. Also considered in the model is the type of donation – whether Source Plasma or for recovered plasma, since the number of donations by an individual per year varies dramatically for these two types of plasma. Finally, the model assumes that *a plasma donation from a vCJD infected individual contains the infectious vCJD agent*, and thus poses a potential risk, if the individual is in the last half of the incubation period for the disease (and likely prionemic). However, current deferral policies prevent potentially infected donors with a history of extended travel or residence to BSE countries since 1980 from donating blood. *This geographic deferral policy effectively lowers the prevalence of vCJD in the US donor population by removing donors with a history of extended travel or residence in the UK and other countries in Europe since 1980.* The FDA risk assessment incorporates the effectiveness of current geographic deferral policy in reducing the risk of vCJD transmission through plasma derivatives. The final outputs or results from the model offer estimates of the potential number of Recovered and Source Plasma donors who may be infected with vCJD, and the model further derives an estimate of the number of infected donors who may actually have agent in their blood at the time of donation.

FDA evaluated a number of possible sources of exposure to the BSE agent that could potentially result in infection with vCJD in US plasma donors. The model and risk assessment assumes that the greatest potential vCJD risk for US plasma donors was likely associated with exposure to the BSE agent during extended travel or residence in the United Kingdom (for 3 months or more from 1980 – 1996), or France and other countries in Europe (5 or more years since 1980).

The following sections in the document describe in detail our mathematical approach for modeling and estimating the risk for US plasma donors who lived or resided in:

- United Kingdom from 1980 – 1996 for ≥ 3 months (Described in Appendix A - section A-IV.C.1.a.)
- France - since 1980 for ≥ 5 years (Described in section IV.C.1.b. and in Appendix A – sections in A-IV.C.1.b.)
- Other countries in Europe – since 1980 for ≥ 5 years (does not include plasma donors) (Described in section IV.C.1.c. and in Appendix A – sections in A-IV.C.1.c.)
- US Military personnel or their dependents – deployed in UK or other countries in Europe since 1980 (Described in section IV.C.1.d. and in Appendix A – sections in A-IV.C.1.d.)
- Euroblood recipients in US – that received blood collected from donors in Europe (Described in section IV.C.1.e. and in Appendix A – sections in A-IV.C.1.e.)

Dietary exposure in the US through consumption of domestic beef potentially contaminated with BSE agent was considered negligible based on our calculations, and was not included in the final model of this risk assessment.

**Assumption used in the model:** The BSE exposure risk for an individual on extended travel or during residence to the UK, France, or other countries in Europe since 1980 is proportional to the duration of the stay or time spent. For instance a person who lived in the UK for one year has one-fifth the risk of a donor who spent five years.

## IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood

The model considered all major potential sources of vCJD infection for US plasma donors. The most likely cause of vCJD is dietary exposure of donors to the BSE agent through the consumption of BSE-contaminated beef during travel to a country where BSE was present in the cattle population. Accordingly, the greatest risk of BSE exposure in the UK likely occurred during the period from 1980 to 1996. The BSE exposure risk for France was likely lower than that of the UK and likely present since 1980. Also, other countries in Europe likely posed an even lower risk than France of human exposure to the BSE agent since 1980. Generally, because of the higher prevalence of BSE in the UK in the late 1980s and early-to-mid-1990s and the higher occurrence of vCJD in the UK human population (currently 162 cases as of August 2006), US donors who traveled to the UK from 1980 through 1996 are likely at higher risk for vCJD infection than donors who traveled to other European countries in the same time period. This model uses the concept of relative risk to estimate the vCJD risk (and prevalence) for a donor population – a value of 1 is used for the UK and this is equal to the vCJD prevalence. Relative risk is used to compare the risk of other regions to that of the UK and is estimated based on factors such as amount of contaminated feed, percentage of meat from the UK, number of cases of BSE, vCJD, etc. In subsequent sections on estimating risk for donors that traveled to France, France is assumed to have a relative risk of 0.05, since they received about 5% of their beef and feed supply from the UK and also have reported domestically-acquired vCJD cases.

The potential vCJD risk faced by US plasma donors exposed to vCJD during travel or residence in the UK (since 1980) is assumed in the model to be proportional to the time a donor spent in the UK (or France or other countries in Europe), and also a function of the age of the donor, and year of travel. Duration of travel is an indicator of possible exposure and we assumed that the probability of exposure was proportional to the time spent in the UK from 1980 - 1996. The longer the duration of travel, the higher the risk of human exposure to the BSE agent. The magnitude of possible exposure to the BSE agent is also influenced by the specific year of travel. The risk is the highest when travel took place during the peak of BSE epidemic in 1992. The FDA risk assessment grouped plasma donors based on age, destination, duration and year of travel, estimated the number of donors, probability of an individual being infected, and potential number of infected donors for each group. Then, numbers of infected donors from all groups were summed to arrive at an estimate of the total number of potential vCJD infected donors in the US that may contribute to FVIII plasma pools. The vCJD agent may only be present in the blood of an infected person during the last half of the disease. Accordingly, the FDA risk assessment considered only those vCJD infected donors who were in the last half incubation period of the disease as being capable of possibly transmitting the disease to others through their plasma donations.

The FDA risk assessment evaluates the effectiveness of current geographic deferral policy in reducing the risk of vCJD transmission through plasma derivatives. Current policy defers individuals with history of long term travel in BSE epidemic areas since 1980, providing a barrier for donors potentially infected, thus effectively lowering the vCJD prevalence in the US donor population by an estimated 90% or more. However, there may be residual risk associated with donors who do not meet criteria for donor deferral, or who meet those criteria, but fail to be deferred due to limitations of the donor screening process. Although current policy defers US donors that received transfusions while in the UK

or France since 1980, the FDA risk assessment did not estimate the potential vCJD risk for these donors.

**Assumption used in the model:** vCJD risk for the US plasma donor population is a sum of the risk from all exposure sources.

**Assumption used in the model:** The FDA risk assessment assumed vCJD agent is present in the plasma of infected person only in the last half of the incubation period of the disease, based on animal studies on vCJD infectivity of blood.

**Assumption used in the model:** The mean incubation period for vCJD is 14 years, and the median incubation period is 13 years.

The United Kingdom has the highest number of reported BSE and vCJD cases in the world. US plasma donors that traveled to the UK during 1980-1996 are considered at risk of exposure to the BSE agent and possibly infected with vCJD. Donors that traveled to France and other countries in Europe are assumed to be at significantly lower risk since the BSE epidemic in those regions was many times smaller than in the UK. This part of the model further characterizes the plasma donors of each age group who have history of travel or residence to the UK (for $\geq$ 3 months from 1980 – 1996), and France or other countries in Europe (for $\geq$ 5 yrs since 1980), by duration and calendar year of travel, estimated the number of potential donors who may be infected, and number of donor who may be in the last half incubation period (prionemic) at year 2002.

## IV. C. 1. a. US plasma donors with history of travel to the UK: Number of donors potentially infected and vCJD agent is present in the blood

This portion of the model calculates the potential vCJD risk for US plasma donors who traveled to the UK since 1980 and estimates the potential number of donors with vCJD agent present in their blood and donated to plasma pools used to manufacture pdFVIII. For blood and plasma donors with a history of travel to the UK FDA guidance (2002) currently recommends that blood and plasma collection centers "....defer blood and plasma donors who have traveled or resided in the U.K. for a cumulative period of 3 or more months between 1980 and the end of 1996." The deferral policy likely eliminates much approximately 90% of the potential vCJD risk associated with donor travel to BSE countries when the disease was present. The model incorporates the effectiveness of the deferral policy in reducing risk. However, residual vCJD risk remains from two sources. One source includes potential risk associated with deferrable donors that continue to donate because of limitations in the donor screening process. The second potential source is associated with donors with a history of travel that is less than the deferrable period (blood or plasma donors with a history of less than 3 months of travel to the UK).

In addition to the effectiveness or deferral policies, the residual risk of vCJD infection in US donors is calculated based on the proportion of time spent in the UK by US donors compared to a UK resident whose risk is equivalent to the UK vCJD prevalence. Additionally, the model considers factors such as calendar year of travel, age of donor, type of donation (Source or recovered plasma), possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US plasma donors who were

potentially infected with vCJD during travel to the UK (see Table 1). The model also predicts the number of donors that potentially have agent present in their plasma that may possibly donate to plasma pools used to manuafacture pdFVIII in the US.

Additional technical information and details on the model calculations of potential vCJD risk and modeling approaches are provided in Appendix A and described in sections in A - IV.C.

## IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

The FDA risk assessment model assumes that the likely source for vCJD risk in US plasma donors is associated with those donors who have a history of travel to the UK from 1980 – 1996, or travel to France or other countries in Europe since 1980. Presumably the greatest vCJD risk resides with travel to the UK since the BSE epidemic was several orders of magnitude larger and the number of vCJD cases greater than that of any other country or region. However, donors who traveled to France are potentially at risk but that risk is likely significantly lower than that of the UK. France likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 5% of its beef was supplied by the UK at the time of its BSE epidemic. To date, France has reported 20 cases of vCJD supporting the notion that there may be vCJD infection risk for US donors that may have traveled to or resided in France since 1980.

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) to estimate potential vCJD risk for donors with a history of travel to countries where BSE has occurred. FDA guidance (2002) indicates that for donors with a history of travel to France "we now recommend deferral of blood and plasma donors with a history of 5 or more years of cumulative residence or travel in France since 1980." This portion of the model calculates the potential residual US vCJD risk based on the estimated vCJD prevalence among US plasma donors who traveled to France since 1980 and donated to plasma pools used to manufacture pdFVIII. The residual vCJD risk includes risk associated with donors who do not meet criteria for donor deferral (e.g., donors with a history of less than 5 years of travel to France), or who meet those criteria, but fail to be deferred due to limitations of the donor screening process. The model considers factors such as duration of travel, calendar year of travel, age of donor, type of donation (Source or recovered plasma), possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US plasma donors potentially infected with vCJD and results are shown in Table 4.4. The model goes on to predict the number of donors with a history of travel to France infected with vCJD that may potentially have agent present in their blood at the time of donation.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of travel to France can be found in Appendix A – in sections under A-IV. C.1.b.

## IV.C. 1. c. Number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) potentially infected and vCJD agent is present in the blood

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) in estimating potential vCJD risk for donors with a history of travel to countries where BSE has occurred. The model quantifies potential residual vCJD risk for US plasma donors. The model assumes that the residual vCJD risk likely originates from two sources, one source of potential risk is associated with donors with deferrable risk that continue to donate due to limitations in the donor screening process. The second source of vCJD risk is associated with donors with a history of travel that was less than the deferrable period (blood donors with a history of less than 5 years of travel to Europe). FDA guidance (2002) indicates that for donors with a history of travel to countries in Europe (other than the UK) "the current recommendation is to exclude from transfusion use, blood and blood components from donors with a history of 5 or more years of residence or travel in Europe outside of the UK". Furthermore, for donors with a history of travel to countries in Europe (other than the UK and France) the FDA guidance (FDA 2002) states "...we do not recommend that you defer Source plasma donors who have lived or traveled in Europe for 5 or more years".

The FDA risk assessment model reflects FDA guidance for vCJD deferral of Source Plasma and recovered plasma donors. Because the guidance recommendations for each type of plasma were different the model estimated the potential vCJD risk as follows:

- Recovered plasma donors – the FDA risk assessment **calculated** the potential vCJD risk because deferral was **recommended** for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK)
- Source Plasma donors - the FDA risk assessment did **not calculate** the potential vCJD risk because deferral was **not recommended** for donors with a history of 5 or more years of residence or travel to countries in Europe (other than the UK and France).

The term "countries in Europe" as used in this portion of the risk assessment is defined as all countries in Europe (other than the UK and France). The UK and France BSE risk is assumed to be higher than that of other countries in Europe, therefore the potential donor vCJD risk for donors with a history of residence or travel to the UK and France described in sections IV.C. 1. a. and IV.C. 1. b.(and sections in Appendix A – under sections A-IV.C. 1. a. and A-IV.C. 1. b.), respectively, of this document. Also, US donors with a history of residence on US military bases in Europe may have a higher potential vCJD risk so their risk was calculated separately and is described in section IV.C.1.d.

This portion of the model calculates the vCJD prevalence, or number of US blood donors who traveled to other countries in Europe (other than the UK and France) since 1980 and were potentially infected with vCJD and donated to recovered plasma pools used to manufacture pdFVIII. Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. The model considers factors such as duration of travel, calendar year of travel, age of donor, type of donation, possible incubation period of the disease, and whether vCJD agent is present in the blood of a vCJD infected donor. The outcome of this portion of the model predicts the number of US blood (recovered plasma) donors who are potentially infected with vCJD and have agent present in their plasma.

The model assumes that one of the most likely sources for vCJD risk in US plasma donors is associated with those donors who have a history of travel to the UK from 1980 – 1996, and France and other countries in Europe since 1980. Text in subsequent sections of this document will specifically discuss the potential vCJD infection risk for US plasma donors with a history of travel to countries in Europe (other than the UK and France) since 1980. Presumably the greatest vCJD risk resides with travel to the UK since 1980 since the BSE epidemic was several orders of magnitude larger and the number of vCJD cases greater than that of any other country or region. France is likely at significantly lower risk than the UK but the risk is still likely higher than the vCJD risk of other countries in Europe. There have been 20 cases of vCJD reported in France as of August 2006.

Donors who traveled to other countries in Europe (other than the UK or France) are potentially at risk but that risk is likely significantly lower than that of the UK, and France. Many European countries (other than the UK and France) likely imported BSE-contaminated feed materials in the 1980s and 1990s and approximately 1.5% of their beef may have been imported from the UK at the time of its BSE epidemic. The potential for BSE exposure to donors who traveled to or resided in other countries in Europe is possible. Hence, there may be a vCJD infection risk for blood donors who may have traveled to or resided in a European country (other than the UK and France) for periods greater than 5 years since 1980. The current US vCJD geographic deferral policy defers blood donors with a history of travel or residence in a country in Europe (other than the UK and France) for 5 years or more since 1980. Source Plasma donors who resided in a country in Europe (other than the UK and France) are not deferred from donation. Because Source Plasma donors are not deferred from donation, their risk is not estimated by the model. Therefore, this portion of the model only estimates potential vCJD risk for US recovered plasma donors who traveled to countries in Europe (other than the UK) since 1980.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of travel to countries in Europe (other than the UK and France) can be found in Appendix A – in sections under A-IV. C.1.c.

## IV. C. 1. d. Number of US plasma donors deployed by the military in the UK or other countries in Europe and potentially infected with vCJD

The FDA risk assessment model incorporates information from current guidance for geographic donor deferrals for vCJD (FDA 2002) in estimating potential vCJD risk for donors with a history of travel to countries where BSE has occurred. The FDA guidance (2002) indicates that for donors with a history of service on US military bases in Europe "we recommend that you should indefinitely defer current and former US military personnel, civilian military personnel, and their dependents who were stationed at European bases for 6 months or more during the time periods outlined (in the document)".

The model quantifies potential residual vCJD risk for deferrable donor populations. The residual vCJD risk likely includes two possible sources of risk. One source of risk is associated with donors with deferrable risk that continue to donate because of limitations in the donor screening process. A second possible source of risk is associated with donors who have a history of travel that is less than the deferrable period (blood donors with a history of less than 5 years of travel to Europe). For the purposes of this risk assessment we assumed that exposure to the BSE agent and potential vCJD infection of military personnel or dependents may have occurred during their deployment to US military bases in the UK, France and other European countries during the period from 1980 through 1996, through consumption of BSE contaminated beef procured for use on US military bases from the UK.

Exposure via UK beef likely varied but the model assumes that up to 35% of beef consumed on military bases in Europe came from the UK. The model assumes that approximately 2% of US blood and plasma donors may have been military, military family or their dependents posted to US military bases in the UK or elsewhere in Europe from 1980 through 1996 (TSEAC, 2002). It was further assumed that the average deployment period was 2 years. Because data on military service of plasma donors was not available, the risk assessment used data available on military deployment by whole blood donors.

Additional technical details on the model calculations of potential vCJD risk for donors with a history of military service in countries in Europe can be found in Appendix A – in sections under A-IV. C.1.d.

## IV. C. 1. e. Annual number of US plasma donors who have been Euroblood recipients

Euroblood is whole blood that was collected at several different collection centers in Europe and shipped to and used by transfusion centers in the United States. The practice was stopped in 2002 with the implementation of geographic vCJD deferrals. The blood was used largely on in the New York City metropolitan area and possibly in other areas on the east coast of the US

The model assumed that a total of 1.2% of US blood donors may have received Euroblood (TSEAC, 2002). To our knowledge there are no specific data available for plasma donors, therefore, data for blood donors was used in this risk assessment.

**Assumption used in the model:** All infected Euroblood recipients have vCJD agent present in their blood and plasma (prionemic)

## IV. C. 1. f. Total number all plasma donors who may potentially be infected with vCJD through all sources of exposure and vCJD agent is present in the blood

This portion of the model sums the total number of all potential US donors that may have been infected with vCJD from different sources. The model estimates the total number of all plasma donors who may be infected with vCJD during extended residence, travel or military service in the UK, France, or other countries of Europe. Potential vCJD risk is also estimated for donors that may have received Europblood. Furthermore, the model estimates the number of total US donors potentially infected with vCJD and have agent present in their plasma.

**Variable:** $\mathbf{DR_{vCJD\text{-}Pn}}$ - Total annual number of all US plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-Pn} = DR_{vCJD-S-Pn} + DR_{vCJD-R-Pn} \qquad \text{(IV.C.1.f-3)}$$

Additional technical information and details of analyses and modeling approaches for estimating potential vCJD in the model are provided in Appendix A under section **A - IV.C.1.**

## IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in the blood and who may not be deferred by questionnaire screening

No validated test is currently available to detect the presence of vCJD agent in blood or plasma. The donor questionnaire, administered to all blood donors, can be used to potentially screen donors for potential vCJD risk based on travel history, specifically involving extended travel to the UK, France or other countries in Europe where BSE was known to occur. In 1999 the FDA implemented a donor deferral policy aimed at reducing the potential risk of donations from those potentially exposed to the BSE agent during extended travel to the UK, France and other countries of Europe. Current policies (FDA 2002) defer blood and plasma donors:

- diagnosed with vCJD or other forms of CJD
- at increased risk for CJD, e.g. the donors have received a dura mater transplant, or human pituitary-derived growth hormone; the donors have blood relatives diagnosed with CJD
- with a history of a 3-month or longer travel/residency period in the UK between 1980-96
- with a history of a 5-year or longer travel/residency period in France since 1980
- current or former US military personnel, civilian military personnel, and their dependents resided in Northern Europe for 6 months or more between 1980-90, or resided in military bases elsewhere in Europe for 6 months or more from 1980 to 1996
- received a transfusion of blood or blood components in the UK since 1980
- injected bovine insulin since 1980 unless it is confirmed that injected bovine insulin was not made after 1980 from UK cattle, and
- whole blood donors with a 5-year or longer travel/residency period in Europe (other than the UK) since 1980

Deferral of donors with a history of travel to BSE countries is an effective tool for eliminating a significant portion of potential vCJD risk in US donors. The model incorporates information on the effectiveness of US deferral policies in reducing potential vCJD risk and potential vCJD prevalence in the US donor population.

**Assumption about variable:** Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Assumption about variable:** Model includes potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) that have long term travel history to the UK (≥3 mo), France (≥ 5 yrs), and Europe (≥5 yrs); and history of military deployment, military dependent or related travel or residence in Europe.

There is a possibility that some individuals that traveled to the UK, France, and other countries in Europe since 1980 stayed for periods of time that were shorter than the deferral period, were exposed to BSE agent, and were infected with vCJD. These individuals represent a source of residual risk – or the remaining risk after interventions (in this case donor deferral policies) are applied. The section below addresses the calculation of residual risk for non-deferred at risk donors that traveled for periods of time that were shorter than recommended guidelines.

The total number of all US plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that may not be deferred by questionnaire screening was determined by summing the estimates generated for both Source and recovered plasma donors that may not be deferred by current screening procedures.

Specific technical information and details of analyses and modeling approaches in estimating the effectiveness of donor deferral policies in reducing potential vCJD risk in US donors are provided in Appendix A under section **A - IV.C.2.**

**Model Results:**
The largest source of potential vCJD risk in US plasma donors is presumably associated with donors who traveled to or resided for extended periods of time in the UK, France and other countries of Europe since 1980. These donors might be exposed to the BSE agent in contaminated beef products and infected with vCJD during travel and residence abroad. Other populations in the US at potential risk for vCJD include US military deployed for extended periods of time in the UK or other countries of Europe and individuals in the US who received blood collected in Europe ("Euroblood"). The prevalence of BSE in the US cattle population is very low and therefore there is a very low probability that domestic dietary exposure to the BSE agent would give rise to human vCJD cases. Because of this very low prevalence, risk via US domestic dietary exposure was assumed to be negligible in the model.

The FDA FVIII risk assessment model uses the concept of relative risk to semi-quantitatively estimate the vCJD risk for US plasma donors with a history of travel to the UK, France and other countries of Europe since 1980. Relative risk is the vCJD risk in a population relative to the UK vCJD relative risk of 1 (or 100%), which is equal to the prevalence of vCJD in the UK. Elements used in the model to calculate vCJD risk for travelers include travel destination (UK, France or other countries of Europe), duration of travel, specific year of travel, and age of donor. The estimated vCJD risk for all potential routes was summed to generate the total mean predicted number of potential vCJD-infected plasma donors in the US. Because of current policies, a blood or plasma donor potentially infected with vCJD has a high probability (85% - 99% chance) of being deferred from donation. Since 2002 FDA has recommended that individuals who resided in the UK for a period of 3 months or more from 1980 to 1996, or resided in France or other countries of Europe for a period of 5 years or more since 1980 be deferred from donating blood or plasma. The model assumes the deferral policy in the US is approximately 85% to 99% effective in reducing the vCJD risk for blood and plasma-derived products. **Table 4.4** (below) shows results from the model predicting the mean number per year of potential vCJD-infected donors and the number of potential vCJD donors who are likely not deferred from donation and donate to plasma pools used to manufacture pdFVIII.

Although it is likely that most would be deferred by the current policy, some plasma donors potentially infected with vCJD may not be deferred and may donate to plasma pools used in the manufacture of pdFVIII. Totaling the estimated number of US donors potentially infected with vCJD yields a mean of approximately 0.01 donors per year based on calculations using a vCJD case-based epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani 2005), or a mean of approximately 1.160 donors per year using calculations based on a tissue sample surveillance study yielding a prevalence estimate of 1 in 4,225 (Hilton *et al* 2004) (**Table 4.4**).

**Table 4.4 Model Results: Annual Number of US plasma donors predicted by model to be potentially infected with vCJD and donate to plasma pools used to manufacture pdFVIII.** Results from model provided for two different UK vCJD prevalence estimates. In the table the mean value is shown above with the 5th and 95th percentiles in parentheses below: The total number of vCJD donors for each prevalence estimate has been rounded to nearest decimal place.

| | **Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on** Clark and Ghani (2005) | | ***Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004)*** | |
|---|---|---|---|---|
| | **Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:** | | **Mean number (5th - 95th perc)[a] US plasma donors with history of travel to:** | |
| | **United Kingdom** | ***France, Europe, or* Military Service** | **United Kingdom** | ***France, Europe, or* Military Service** |
| ***Total number vCJD donors for all US pdFVIII pools Prior to screening*** | *0.0493* (0–0)[b] | *0.0108* (0–0)[b] | *5.32* (0–13) | *0.77* (0–3) |
| **Number vCJD donors NOT DEFERRED (ineffective screening)** | *0.0035* (0–0)[b] | *0.0007* (0–0)[b] | *0.39* (0-2) | *0.056* (0–0)[b] |
| ***Number vCJD donors NOT DEFERRED (short-term travel <3 mos. UK; <5 yrs. FR and EU)*** | *0.0049* (0–0)[b] | 0.0017 (0–0)[b] | *0.483* (0–2) | 0.0343 (0–0)[b] |
| ***Total number vCJD infected donors NOT DEFERRED Donate to pdFVIII Plasma Pools*** | ***0.013*** (0–0)[b] | | ***1.160*** (0–4) | |

[a] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## IV. D. Annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US

*Model Results:*
The percentages of source or recovered plasma pools, potentially containing vCJD agent, used to manufacture pdFVIII in the US were estimated by the model. The majority of pdFVIII is manufactured from Source Plasma and the minority from recovered plasma. Manufacturers provided information to FDA on the approximate range and average number of donations per plasma pool which was combined with information on market share to develop two aggregate statistical distributions, one each representing donations for source and for recovered plasma pools. The distributions were used to predict the number of donations per source or recovered plasma pool in the model. The model used information on the number of donations per pool by type (either source or recovered), combined with estimated yield of pdFVIII per pool, and estimated the total number of plasma pools used to manufacture pdFVIII products distributed in the US in 2002.

As a general comment, the number of donations per plasma pool influences the potential exposure risk for infrequent recipients of plasma derivatives. The use of fewer donations and smaller plasma pools during manufacturing would result in a lower percentage of plasma pools potentially containing vCJD agent and potentially expose a lower percentage of infrequent recipients to vCJD (if present). Frequent recipients of plasma-derived products would likely face a similar level of risk of potential vCJD exposure whether large or small numbers of donations per plasma pool are used in manufacturing.

*Lower UK vCJD prevalence estimate of ~1.8 in 1,000,000 (based on Clarke and Ghani, 2005).* The lower prevalence estimate used in the FDA model suggested that an average of 0.027% of all US plasma pools used to manufacture pdFVIII in the year 2002 potentially contained the vCJD agent (bottom, **Table 4.5**). The lower disease prevalence is associated with model results predicting a much lower percentage of plasma pools potentially containing vCJD agent. In fact, on average >99.9% of the time plasma pools would be predicted not to contain a donation from a vCJD infected donor. Only an average 0.10% recovered plasma pools would be predicted by the model to contain a vCJD donation from a US donor in any given year. Of interest at the lower prevalence, the model predicts that the occurrence of a recovered plasma pool with a vCJD donation would be infrequent (as indicated by 5$^{th}$ and 95$^{th}$ percentile values of 0); occurring (as estimated by the model), at a rate of 1 in 100 years. Also at the lower prevalence, a vCJD donation in a Source Plasma pool would be predicted to be even more infrequent and predicted by the model to occur at a rate of 1 in 200 years.

*Higher UK vCJD prevalence estimate of 1 in 4,225 (Hilton et al 2004).* The higher prevalence estimate used in the FDA model suggested that an average of 2.41% of all US plasma pools used to manufacture pdFVIII in 2002 were predicted by the model to contain vCJD agent (**Table 4.5**). It should be noted that fewer recovered plasma pools than Source Plasma pools are used in the US annually to produce pdFVIII. Also, recovered plasma pools contain the largest number of plasma donations. Since recovered plasma pools

contain many more donations than Source Plasma pools the likelihood that a recovered plasma pool may contain a donation from an individual potentially infected with vCJD is considerably higher than for a Source Plasma pool. Using the higher UK vCJD prevalence estimate, the model predicts that on average, 9.12% of recovered pools and 0.96% of Source Plasma pools potentially contain vCJD agent.

**Table 4.5 Annual Percentage of US Plasma Pools Potentially containing a vCJD Donation.** Results from model include only those US plasma pools used annually to manufacture pdFVIII.

- Results provided for two different UK vCJD prevalence estimates.

| | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004) | |
|---|---|---|---|---|
| | Source Mean ($5^{th}$ - $95^{th}$ perc)[a] | Recovered Mean ($5^{th}$ - $95^{th}$ perc)[a] | Source Mean ($5^{th}$ - $95^{th}$) perc)[a] | Recovered Mean ($5^{th}$ - $95^{th}$ perc)[a] |
| **Percent pools potentially containing vCJD agent** | 0.01% (0 – 0%)[b] | 0.10% (0 – 0%)[b] | 0.96% (0 – 5.88%) | 9.12% (0 – 40.17%) |
| **Average percent pools potentially containing vCJD agent** | **0.027 %** (0 – 0 %)[b] | | **2.41 %** (0 – 10 %) | |

[a] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[b] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

Additional technical details on the calculation of the annual total percentage of all plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US are provided in Appendix A in sections under A-IV. D.

## IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD

Based on limited available data (see below), FDA believes that the quantity of infectivity present in blood from a vCJD infected individual in i.v. $ID_{50}$ is likely represented by a distribution with the following characteristics: Minimum value = 0.1, $5^{th}$ percentile = 2,

Most likely value = 10, 95$^{th}$ percentile = 30, and Maximum value= 1,000 i.v. $ID_{50}$. Given the possible parameters, statistical distributions were fitted to the selected parameters using Best Fit part of the @Risk Professional software package (Palisade Corporation, New York). Using the software we determined that a log normal statistical distribution of (2, 12, 30) i.c. $ID_{50}$/ml (5$^{th}$ percentile, most likely, and 95$^{th}$ percentile) with minimum and maximum of 0.1 and 1,000, respectively, provided the best fit.

Conclusions from several research groups arrive at somewhat similar estimates for the quantity of infectivity that might be present in the whole blood of mice and hamsters. Using a mouse model and human CJD Brown *et al* (1999) found a range from 0.5 to 15 mouse i.c. IU per ml which we assumed to be roughly equivalent to 1 to 30 i.c. $ID_{50}$ (assuming a linear dose-response for infectivity). An infectious unit is the quantity of infectivity associated with a 100% probability of infection in recipients and roughly equates to two $ID_{50}$ units (1 IU = 2 $ID_{50}$). Brown *et al* (1998, 1999) conducted experiments to determine the infectivity of buffy coat material and plasma but not red blood cells. Assuming that red blood cells retain approximately 25% of the infectivity of whole blood, then the infectivity present in whole blood could be estimated to be in the range of approximately 10 i.c. $ID_{50}$ and 20 i.c. $ID_{50}$ per ml. Cervenakova *et al* (2003) found levels as high as 20 – 30 infectious doses per ml (40-60 i.c. $ID_{50}$ per ml) associated with buffy coat and plasma during incubating and symptomatic stages of the disease. Red blood cells were not found to be infectious. Transfusion of blood products using the hamster scrapie model by Rohwer suggests that addition of infectivity levels derived for individual blood components would generate a titer for whole blood of approximately 2 to 20 i.c. $ID_{50}$/ml. Summarizing the above literature it seems that the range of reported values for infectivity ranged from 0.5 to as high as 30 i.c. $ID_{50}$ with the possibility that at times the infectivity present in blood may exceed this range.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5$^{th}$ percentile of 2 i.c. $ID_{50}$ per ml, a medium of 12 i.c. $ID_{50}$ per ml, a 95$^{th}$ percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected(Wadsworth, 2001).

## IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD

This section of risk assessment estimated quantity of vCJD agent in each vCJD plasma pool that may be used to make pdFVIII. Quantity of infectious agent present in plasma

pools may vary depending on number of infected donations in the pool and the volume of each infected donation.

**Variable:** $I_{bl}$ – Represents the i.c. $ID_{50}$ present in the blood of individual infected donor ($ID_{50}$/ml) in the last half of the incubation period of vCJD.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5$^{th}$ percentile of 2 i.c. $ID_{50}$ per ml, a median of 12 i.c. $ID_{50}$ per ml, a 95$^{th}$ percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected (Wadsworth, 2001).

**Variable:** $I_{pl\text{-}perc}$ – Percent (%) i.v. $ID_{50}$s associated with plasma

Studies in animal models have shown that greater than 50% of transmissible spongiform encephalopathy agent present in whole blood is associated with plasma. Experiments by Gregori *et al.* (2004) using a hamster – sheep scrapie model showed that approximately 58% of infectivity in whole blood is associated with plasma.

**Assumption used in the model:** The model assumes that 58% of infectivity is associated with plasma.

Studies with mouse-adapted scrapie agent suggest that the i.v. route of administration is approximately 10 times less efficient in causing infection than the intracerebral route (Kimberlin *et al* 1996). Brown *et al* (1999) used a mouse-adapted human TSE agent to show that i.v. injection of plasma was about seven times less efficient and i.v. injection of buffy coat approximately 5 times less efficient than were i.c. inoculations of the same materials in transmitting infection. Based on discussion and advice from the FDA Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC, 2005) the range of efficiency of the i.v. route (versus the i.c. route) was assumed in the model to range between the values of 1 and 10.

**Assumption used in the model:** Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

## IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD

The quantity of vCJD agent potentially present in a donation from a US donor infected with vCJD will be diluted out in a plasma pool among plasma from thousands of other donations. This section calculates the quantity of agent potentially present in a plasma pool potentially containing a donation that contained vCJD agent.

**Assumption used in the model:** We assumed only one infected donor per plasma pool, because based on the calculation in section IV. C. 5. the prevalence of vCJD in the US is very low and the chance a pool involves multiple donations from vCJD infected donors is small.

**Variable:** $DN_{DR}$ - Number of donations from an infected plasma donor, which varies based on type of plasma donated.

**Assumption used in the model:** We assumed individual infected Source Plasma donor most likely give [redacted] donations to a pool, with minimum of 1, maximum of 12 donations. Individual infected recovered plasma donors most likely give only one donation to a pool.

**Variable:** $I_{Pool}$ - Initial infectivity in an infected plasma pool is represented by the equation:

$$I_{Pool} = I_{DN} \times DN_{DR} \qquad \text{(IV.E.2-1)}$$

## Module 3: Reduction in the quantity of vCJD agent during manufacture of pdFVIII

The plasma separated from whole blood is a protein rich, straw-colored liquid that contains FVIII, a number of other clotting factors, immune globulins, serum albumin and a number of other proteins. Individual proteins, such as FVIII, can be purified by dividing or fractionating the plasma into its various protein components. Fractionation steps may include alcohol precipitation, size exclusion, affinity chromatography, etc. The quantity of vCJD agent present in plasma-derived products may be reduced through removal of the agent during the fractionation process. Although the quantity of agent may be significantly reduced, it may not be entirely eliminated.

Common viral inactivation procedures such as heating, solvent-detergent treatment, and UV irradiation have little effect on the quantity of TSE infectivity present in plasma and plasma derivatives. However, several research studies suggest that TSE infectivity in plasma may be partially removed during the fractionation process. The plasma fractionation procedures, which may remove vCJD infectivity, are summarized in Table 4.6. (Lee et al. 2000; Stenland *et al.* 2002; Foster 2004; Foster, *et al.* 2004).

For a specific pdFVIII product, usually only one or two processing steps have been studied for potential reduction of infectivity. Experimental designs of these studies are not standardized; therefore, study results are not directly comparable. In order to achieve a high concentration of vCJD infectivity in initial materials, many studies used vCJD infected brain homogenate as spiking material, which may not mimic the physical form of infectious agent in the blood. Considering the uncertainty in the degree of reduction that can be achieved during various pdFVIII manufacturing processes, this risk assessment models three levels of potential reduction in infectivity, 2-3 $\log_{10}$, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

**Table 4.6 Reduction factor (RF) of fractionation procedures**

| Fractionation Procedures | RF ($\log_{10}$ $ID_{50}$) | References |
|---|---|---|
| Cryoseparation | 0-1 | (Foster *et al* 2000; Farrugia 2002; Lee *et al* 2002) |
| General fractionation | | |
| 3% PEG | 1-3 | (Farrugia 2002; Lee *et al* 2002) |
| 11.5% PEG | 2-4 | (Farrugia 2002) |
| $Zn+Al(OH)_3$ | 1.7 | (Foster *et al* 2000) |
| Ion exchange | 2.7-3.5 | (Foster *et al* 2000; Cervenakova *et al* 2002) |
| Membrane filtration | 1 | (Foster *et al* 2000) |
| Immunopurification | 4.4-6.3 | (Foster, Welch *et al* 2000; Cervenakova *et al* 2002) |

## IV.E. 3. Model results: Estimates of the per vial vCJD infection risk for US manufactured pdFVIII (Module 3)

The mean potential risk of vCJD infection per 1,000 IU vial of US manufactured pdFVIII is shown in **Table 4.7.** The mean potential per vial vCJD risk per year is a function of two factors:

1) Percentage of pdFVIII vials containing vCJD agent and,
2) Quantity of agent (i.v. $ID_{50}$) present in each vial.

If vCJD agent were present in US plasma pools, the risk assessment model assumed that the quantity of agent was likely reduced by manufacturing processes used to produce purified pdFVIII. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have 4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. **Table 4.7** shows potential risks associated with products attaining a 4-6 $\log_{10}$ level of reduction during manufacture. Results are shown only for 1,000 IU vials but the model assumed that the final purified pdFVIII product was packaged with equal likelihood into vial sizes of 250, 500, 1,000 and 1,500 international units (IU).

*Per vial vCJD risk: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani, 2005).* The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the lower prevalence estimate *(based on Clarke and Ghani 2005)* and results are shown in **Table 4.7.** Based on the lower prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 0.027%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. $ID_{50}$ per vial is $3.59 \times 10^{-5}$ for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 55,710 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 210 million. Alternatively, this could be taken to mean that a patient would need to infuse 210 million vials of product before accumulating one full infectious dose of vCJD.

At this lower prevalence estimate, there is a lower probability that plasma pools contain a donation from a donor potentially infected with vCJD, and a pdFVIII vial would be much less likely to contain vCJD agent. Although the probability (0.027%) is lower, the mean quantity of vCJD agent (iv $ID_{50}$) and risk of exposure to vCJD agent (1 in 55,710) per vial derived from a pool containing a single vCJD donation would likely be unaffected by prevalence. Readers may notice that the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) are from 0 to 0, meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. Again, the model predicts that, on average, 0.027% of the time the exposure to vCJD may be greater than zero.

*Per vial vCJD risk: Results based on higher surveillance prevalence estimate of 1 in 4,225 (Hilton, et al 2004).* The per vial risk provides an estimate of the potential vCJD infection risk for a 1,000 IU vial of pdFVIII product manufactured from plasma collected from US donors. The model generated estimates of per vial risk using the higher prevalence estimate (Hilton, et al 2004) and results are shown in **Table 4.7.** Using the higher prevalence estimate the average percent of plasma pools containing the vCJD agent is estimated to be 2.41%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. $ID_{50}$ per vial is $3.59 \times 10^{-5}$ for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 55,710 vials made from a contaminated pool would contain an infectious dose of vCJD. Combining these estimates yields an average risk per vial of 1 in 2.3 million. Alternatively, this could be taken to mean that a patient would need to infuse 2.3 million vials of product before accumulating one full infectious dose of vCJD. Again, although the probability (2.41%) is higher at the higher prevalence, the mean quantity of vCJD agent (iv $ID_{50}$) and risk of exposure to vCJD agent (1 in 55,710) per vial derived from a pool containing a single vCJD donation would likely be unaffected by prevalence.

**Table 4.7 Annual Predicted per Vial vCJD Infection Risk for US Manufactured pdFVIII from Model:**

- Results for 1,000 IU vial
- Assumed manufacturing process reduction of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

| | 4 - 6 $\log_{10}$ Reduction | | | | | |
|---|---|---|---|---|---|---|
| | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004) | | |
| Type of Plasma Pool | Percentage FVIII vials with vCJD agent ($5^{th}$, $95^{th}$ perc)[c] | Quantity iv $ID_{50}$[a] per vial* ($5^{th}$- $95^{th}$ perc)[c] | Mean potential per vial vCJDrisk[b] ($5^{th}$- $95^{th}$ perc)[c] | Percentage FVIII vials with vCJD agent ($5^{th}$, $95^{th}$ perc)[c] | Quantity iv $ID_{50}$[a] per vial* ($5^{th}$- $95^{th}$ perc)[c] | Mean** potential per vial vCJDrisk[b] ($5^{th}$- $95^{th}$ perc)[c] |
| Source | 0.01% (0 – 0%)[d] | $4.36 \times 10^{-5}$ ($4.51 \times 10^{-6}$ – $1.49 \times 10^{-4}$) | 1 in 459 million (0, 0)[d] | 0.96% (0 – 5.88%) | $4.36 \times 10^{-5}$ ($4.51 \times 10^{-6}$ – $1.49 \times 10^{-4}$) | 1 in 4.6 million (0, 1 in 238,000) |
| Recovered | 0.10% (0 – 0%)[d] | $2.56 \times 10^{-6}$ ($3.13 \times 10^{-7}$ – $8.12 \times 10^{-6}$) | 1 in 800 million (0, 0)[d] | 9.12% (0 – 40.17%) | $2.56 \times 10^{-6}$ ($3.13 \times 10^{-7}$ – $8.12 \times 10^{-6}$) | 1 in 8.6 million (0,1 in 813,000) |
| Average of all vials | 0.027 % (0 – 0%)[d] | $3.59 \times 10^{-5}$ ($8.18 \times 10^{-7}$ – $1.29 \times 10^{-4}$) | 1 in 210 million (0, 0)[d] | 2.41 % (0 – 10 %) | $3.59 \times 10^{-5}$ ($8.18 \times 10^{-7}$ – $1.29 \times 10^{-4}$) | 1 in 2.3 million (0, 1 in 155,000) |

*Mean i.v. $ID_{50}$ in vials containing vCJD agent

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual per vial vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Percentage vials with vCJD agent x mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation

This section of the risk assessment models each infected plasma pool to estimate the potential reduction in infectivity for each pool during manufacturing processing, and to estimate the quantity of any remaining infectivity (I,v, $ID_{50}$s) in the pdFVIII product made from each pool.

The levels of reduction of vCJD agent achieved during manufacturing and processing varies among the pdF VIII products on the market. To our knowledge, the total level of reduction in vCJD agent achieved during manufacturing for any pdFVIII product on the market is not known. However, the levels of TSE clearance and reduction for some of the individual fractionation steps (or similar steps) used in the manufacture of some pdFVIII products is known. Based on TSE clearance studies in the published literature and manufacturers' data available to the FDA, FDA staff believe that the plasma-derived products currently on the market employ manufacturing processes that achieve a clearance of vCJD agent of 4 $\log_{10}$ or greater in the final pdFVIII product. The FDA model employed three stratifications of clearance:

- 2 – 3 $\log_{10}$
- 4 – 6 $\log_{10}$
- 7 – 9 $\log_{10}$

Each of these levels of clearance was modeled separately. Most of the results are presented for the 4-6 $\log_{10}$ reduction during manufacture processing in the risk characterization section (Section V.) of this risk assessment.

## Module 4: FVIII utilization and annual exposure

### *Background*

Patients with hemophilia A (HA) have an inherited – recessive, sex-linked bleeding disorder that affects approximately 14,000 individuals in the United States (Soucie *et al* 1998). FDA estimated that there are approximately 1,800 patients in the US with severe disease who use plasma-derived products. The blood of affected individuals contains functionally abnormal or abnormally low concentrations of FVIII. FVIII is a protein in blood plasma that is part of the blood coagulation pathway and is critical for the normal clotting of blood. In the case of severe disease, FVIII is <1% of normal. Among severely affected persons, spontaneous bleeding or bleeding at the site of an injury or a joint is common and can lead to severe disability or death without treatment. The complications of HA can be prevented by appropriate clinical management and treatment with pdFVIII or recombinant FVIII products.

Traditionally, HA patients were treated with factor concentrates only when bleeding occurred, which is called episodic treatment. Currently, the adoption of a preventative form of treatment or prophylaxis therapy is increasing. Prophylactic therapy refers to treatment regimens that seek to prevent bleeding events with regular infusions of clotting factor concentration. Prophylactic treatment greatly decreases the likelihood of adverse events such as joint hemorrhage, and effectively prevented the development of chronic joint arthropathy and disability, but requires higher use of FVIII product than episodic treatment.

Patients with vWD have an inherited, non-sex linked bleeding disorder associated with abnormal platelet adhesion caused by deficiency in von Willebrand Factor (vWF) activity. FDA estimated that there are approximately 250 patients in the US with severe vWD who use plasma-derived products. Mucosal bleeding is common in patients with vWD due to the platelet adhesion disorder. In some cases there may be a deficiency in FVIII as well. Patients with severe vWD can experience persistent bleeding into joints resulting in pain, degeneration of joints, swelling and loss of range of motion similar to patients with HA. Mild forms of vWD are often treated successfully with desmopressin but more severe forms of the disease usually necessitate treatment with coagulation factor concentrates that contain both vWF and FVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

## IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII

The potential exposure of an individual HA or vWD patient or patient population with severe disease to the vCJD agent through use of pdFVIII was estimated in the model based on the:

- total quantity of pdFVIII used per year, and
- potential quantity of vCJD agent predicted in the pdFVIII product.

## IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients

The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen. Plasma-derived FVIII utilization and the size of each of the severe HA clinical treatment subpopulations were estimated using data from a Centers for Disease Control (CDC) sponsored study by 6 states by Hemophilia A patients from 1993-1998. This risk assessment provides outputs that estimate the annual exposure for several patient subpopulations with Severe HA disease for patients in the following clinical treatment groups:

- Prophylaxis – No inhibitor
- Prophylaxis - With inhibitor
- Prophylaxis - With inhibitor and immune tolerance

- Episodic – No inhibitor
- Episodic - With inhibitor

Because patients with severe HA are likely to use higher quantities of pdFVIII product we reasoned that they would be at potentially greater risk, than those with moderate or mild hemophilia, if the vCJD agent were present in US manufactured product.

A summary of the utilization data used for the model are provided in the table below. Additional technical details of the analysis of FVIII utilization for this population are described in section A-IV.G. of Appendix A.

**Table 4.8. Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution**

| | | Input distribution | | | |
|---|---|---|---|---|---|
| **Treatment Regimen** | **Inhibitor Status** | **n** | **(min, max)** | **Mean** | **95% CI** |
| *Prophylaxis* | No Inhibitor | 578 | (300, 1200000) | 157949 | (21242, 282316) |
| | With Inhibitor – No Immune Tolerance | 63 | (2000, 800000) | 190523 | (26856, 447639) |
| | With Inhibitor – With Immune Tolerance | 62 | (100000, 2000000) | 558700 | (33235, 1592943) |
| *Episodic* | No Inhibitor | 946 | (0, 1000000) | 85270 | (4633, 244658) |
| | With Inhibitor | 151 | (2200, 1000000) | 180458 | (5314, 488906) |

## IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients

The CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 did not include patients with vWD. We assumed that vWD patients with severe disease would largely use Humate P product only for factor replacement treatment. A search of records in the Hemophilia Surveillance System project data revealed a total of 58 records that indicated Humate P had been used, among which, 8 records indicates patients had developed inhibitor, which are considered uncommon among vWD patients and were excluded from analysis. Among the 58 records, 35 were from Adults (≥15 yrs of age) and 23 records were from young persons (<15 yrs of age). Records for each age group were further grouped by clinical treatment using either a prophylaxis or episodic treatment regimen. Data were initially analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate descriptive statistics and statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. The Generalized Beta distribution was identified as the best fit to the pdFVIII utilization data (as determined by using the software Best Fit (Palisade Corp, NY) and was used as the input distribution for pdFVIII usage by individual vWD patients in the model. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. Table 4.9. summarizes pdFVIII usage data from CDC sponsored study and the input distribution generated based on the data. FDA used data in the CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 to estimate FVIII utilization by all vWD patients. The data represent only a sample of all possible vWD patients with severe disease in the US. FDA estimated that there were approximately 250 patients in the US with Type 3 vWD. To calculate the total number of patients in each age group and treatment regimen group we adjusted the 58 patient population to equal a total of 250 patients by multiplying the patient population in each group by a factor of 4.3 (250/58 = ~4.3). The utilization data for patients in each treatment regimen in the sample population were used in the risk assessment model to generate outputs for the annual exposure to vCJD for all vWD for Adult (>15 yrs of age) and Young (≤15 yrs of age) persons in the US among clinical treatment groups of prophylaxis and episodic. The FVIII utilization data were used to calculate the potential vCJD risk for vWD patients; these results are shown in Tables 5.2A and 5.2B.

**Table 4.9. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution**

| Treatment Regimen | n | (min, max) | Mean | 95% CI |
|---|---|---|---|---|
| **Young** (<15 yrs of age) | | | | |
| *Prophylaxis* | 9 | (9200, 504625) | 165713 | (9346, 479457) |
| *Episodic* | 14 | (1010, 41850) | 11045 | (1013, 37543) |
| **Adult** (>15 yrs of age) | | | | |
| *Prophylaxis* | 17 | (15000, 772800) | 186880 | (15570, 606670) |
| *Episodic* | 18 | (1000, 293800) | 86923 | (1362, 260660) |

# V. RISK CHARACTERIZATION

The risk characterization section of the risk assessment integrates information from the hazard identification, hazard characterization and the exposure assessment components to arrive at estimates of the risks posed by a hazard.

In this risk assessment data for hazard characterization are lacking, so we could not develop a human vCJD dose-response. The dose-response relationship provides information needed to use the exposure (dose) assessment results to estimate the probability of adverse responses including infection, illness or mortality – based on assessment of exposure (dose) to the hazard. Many TSE models and risk assessments, including our model, use the $ID_{50}$, or amount of material that leads to infection in 50% of the population, as a semi-quantitative estimate of the amount of TSE agent. The $ID_{50}$ has been derived from rodent animal models and may or may not approximate infection and occurrence of vCJD in humans. This lack of knowledge about the animal data and how they relate to actual human clinical vCJD outcomes adds considerable uncertainty to the risk estimates generated by the model. The FDA risk assessment interprets the $ID_{50}$ as representing a linear dose-response relationship or linear relationship between exposure and the probability of infection. In such a case exposure to 1 $ID_{50}$ would suggest a 50% probability of infection, exposure to 0.1 $ID_{50}$ would suggest a 5% probability of infection, and so on.

The final results of this risk assessment provide estimates of potential annual exposure and annual vCJD infection risk for patients with severe HA and for patients with severe vWD for pdFVIII manufactured from plasma collected in the US. The risk was estimated by applying the linear $ID_{50}$ dose-response relationship, which provides a probability of vCJD infection in the two populations and various subpopulations within the two groups. Given the limited data available FDA believes that any extrapolation or interpretation has limited utility in actually estimating outcomes such as infection and illness. Therefore, any estimate of the risk based on estimates of exposure to the vCJD agent through use of pdFVIII will be imprecise and extremely uncertain.

## V.A. THE MODEL

This risk assessment and simulation model links the available scientific and epidemiological data together to mathematically approximate the processes (predicted presence of vCJD in UK population, manufacturing, reduction of vCJD agent, and patient utilization) leading to potential exposure of US patients to vCJD agent present in US-manufactured pdFVIII. A summary of the variables, parameters and equations used in the model were described in Section III. Exposure Assessment and a summary of the variables and equations, data, and assumptions used in the model are provided in Appendix A. The model was run using @Risk software package (Palisades Corp, NY) to conduct the Monte Carlo analysis. Simulations of 10,000 iterations were run.

The risk assessment uses Monte Carlo simulation to randomly draw values from probability input distributions (which are statistical representations of input data) once per iteration; thousands of iterations are used to generate the model outputs as risk estimates. This simulation method is often used in situations when a model is complex, non-linear, or involves several uncertain parameters. The output generated is usually an aggregate distribution whose shape can be summarized using measures of central tendency (mean, median, mode) or with boundaries such as the 95% confidence interval (CI), the $5^{th}$ and $95^{th}$ percentiles (representing the 90% CI) or the range, bounded by the minimum and maximum values generated as part of the output. The strength of Monte Carlo analysis is that it generates resulting risk estimates as statistical distributions, which reflect the underlying uncertainty and variability of the original input data and parameters.

## V. B. Model results: Estimated annual potential exposure to vCJD i.v. $ID_{50}$ and potential vCJD risk through human pdFVIII used to treat severe HA

Individuals with HA vary in their degree of FVIII deficiency. Although the clinical spectrum generally can range from severe, to moderate, and to mild disease, this assessment specifically addresses potential vCJD exposure and risk for persons with severe HA. Among an estimated 14,000 HA population in the United States, approximately 50% have severe disease and 25% of all HA patients use human pdFVIII products. FDA

estimated that there are a total of approximately 1,800 HA patients (**Tables 5.1A. and 5.1B.**) with severe disease in the US that use human pdFVIII products. Although the estimated risk is very low, it is possible that some patients using human pdFVIII may potentially be exposed to vCJD agent if present in US manufactured product.

*Estimation of PdFVIII product utilization by patients with severe HA.* FDA obtained data on human plasma-derived FVIII utilization from the Centers for Disease Control (CDC). Data in the study were collected as part of a collaborative effort between CDC and six states during the time period 1993 – 1998. A summary of study results for New York State are described in Linden, et al. (2003). The comprehensive study collected standardized patient demographic, clinical, treatment and outcome data. Patient medical records were obtained from treatment sites including: hemophilia treatment centers (HTCs), hospitals, clinics, physician's offices, home-care agencies, nursing homes, prison infirmaries, and dispensers of factor concentrates. The data abstracted from medical records tabulated all factor concentrate utilization prescribed by quantity, type, purpose (e.g., prophylaxis, treatment of acute bleeds, or immune tolerance therapy) and total quantity used per calendar year.

The data on quantity of pdFVIII product utilized annually were used to develop statistical distributions of product usage for patients by treatment group. The mean quantities of products utilized by HA patients on different treatment regimens are shown in **Table 5.1A. and 5.1B.** Approximately 1,100 records for patients utilizing pdFVIII were analyzed in this study. The percentage of each patient subpopulation in proportion to the total HA population in the CDC-Six State study was used to extrapolate the estimated number of total individuals in each patient subpopulation. From the study results, we estimated that there are a total of approximately 1,800 persons with severe HA in the US who use pdFVIII.

Results from the risk assessment model for patients with severe HA who are treated with pdFVIII product with a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in **Tables 5.1A. and 5.1B.** Generally results are expressed for patients in several different HA clinical treatment groups including:

- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

*Potential exposure of severe HA patients to vCJD agent: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (based on Clarke and Ghani, 2005).* The model estimates that severe HA patients treated using a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) has the highest pdFVIII usage of the groups we examined and potentially face the highest risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year and are potentially

exposed to an average of $1.57 \times 10^{-6}$ i.v. $ID_{50}$ per person per year; representing an average potential vCJD risk of 1 in 1.3 million per person per year. If all of the assumptions in the model are correct at this lower estimated prevalence, this risk may yield 1 vCJD infection in an average of approximately 21,000 years of treatment among severe HA patients who are in a prophylaxis treatment regimen with inhibitor and immune tolerance. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) in **Table 5.1A.** are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicts that 0.027% of the time the exposure to vCJD agent may be greater than zero, and there is a possible but low risk of vCJD infection.

The risk for the entire population is calculated by summing the cumulative risk potential of vCJD exposure and risk **(Table 5.1B.)**. Using the lower prevalence estimate, the model predicts that the approximately 1,800 severe HA patient population in the US uses a total of approximately 243 million IU pdFVIII and is exposed to an average of $6.50 \times 10^{-4}$ i.v. $ID_{50}$. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential population-based vCJD risk of 1 in 3,077. At this expected level of risk, 1 vCJD infection would be predicted to occur in 3,077 years of treatment for the entire population of 1800 severe HA patients that use pdFVIII.

*Potential exposure of severe HA patients to vCJD agent: Results based on higher surveillance prevalence estimate of 1 in 4,225 (Hilton, et al 2004).* The model estimates that severe HA patients in a prophylaxis regimen, with inhibitor, with immune tolerance and treated with a pdFVIII product (with 4-6 $\log_{10}$ reduction of vCJD agent) potentially face the highest expected risk among HA patients. **Table 5.1A.** indicates that approximately 62 severe HA patients in a prophylaxis treatment regimen with inhibitor and immune tolerance use an average of 558,700 IU per person per year, and are potentially exposed to an average of $1.30 \times 10^{-4}$ i.v. $ID_{50}$ per person per year, using the higher prevalence estimate. This represents an average potential vCJD risk of 1 in 15,000 per person per year for the treatment group. If all of the assumptions used in the model are correct and considering the total number of 62 patients in this category (or population-based risk), this expected risk would yield 1 vCJD infection in 240 years of treatment among the patients under this category.

The risk for the entire severe HA population is calculated by summing the cumulative risk potential of vCJD exposure and risk from all individual patients under five categories (prophylaxis with no inhibitor, prophylaxis with inhibitor, prophylaxis with inhibitor and immune tolerance, episodic with no inhibitor and episodic with inhibitor) **(Table 5.1B.)**. Using the higher surveillance estimate, the model predicts that the approximate total of 1,800 severe HA patient population in the US uses a total of approximately 243 million IU pdFVIII, and is exposed to an average of $5.67 \times 10^{-2}$ i.v. $ID_{50}$ per year. This total annual exposure for the entire severe HA population in the US is equivalent to a mean potential

population-based vCJD risk of 1 in 35, i.e., 1 vCJD infection would be predicted to occur in 35 years of treatment in this 1800 severe HA patient population.

**Table 5.1A. Model Results for All HA Patients who use a Hypothetical Factor VIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent: *Predicted Annual per Person Exposure to vCJD i.v. $ID_{50}$ and Mean Potential per Person Annual vCJD Risk:***

- For patients with SEVERE disease, and
- Two different UK vCJD prevalence estimates.

| | | | | 4 - 6 $Log_{10}$ Reduction | | | |
|---|---|---|---|---|---|---|---|
| | | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | |
| Treatment Regimen | Inhibitor Status | Est. Total Number patients in US | Mean quantity FVIII used per person per year ($5^{th}$ - $95^{th}$ perc) | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] |
| *Prophylaxis* | No Inhibitor | 578 | 157949 IU[d] (21242 , 382316 ) | $4.99\times10^{-7}$ (0-0)[e] | 1 in 4.0 million (0-0)[e] | $3.67\times10^{-5}$ ($0 - 1.72\times10^{-4}$) | 1 in 54,000 (0 - 1 in 12,000) |
| | With Inhibitor – No Immune Tolerance | 63 | 190523 IU[d] (26956, 447639) | $4.21\times10^{-7}$ (0-0)[e] | 1 in 4.8 million (0-0)[e] | $4.86\times10^{-5}$ ($0 - 2.17\times10^{-4}$) | 1 in 41,000 (0 - 1 in 9,000) |
| | With Inhibitor – With Immune Tolerance | 62 | 558700 IU[d] ( 33235, 1592943) | $1.57\times10^{-6}$ (0-0)[e] | 1 in 1.3 million (0-0)[e] | $1.30\times10^{-4}$ ($0 - 5.39\times10^{-4}$ ) | 1 in 15,000 (0 - 1 in 3,700 ) |
| *Episodic* | No Inhibitor | 946 | 85270 IU[d] ( 4633, 244656) | $2.12\times10^{-7}$ (0-0) | 1 in 9.4 million (0-0)[e] | $1.91\times10^{-5}$ ($0 - 8.50\times10^{-5}$) | 1 in 105,000 (0 - 1 in 24,000 ) |
| | With Inhibitor | 151 | 160458 IU[d] (5314, 488906 ) | $2.49\times10^{-7}$ (0-0)[e] | 1 in 8.0 million (0-0)[e] | $4.19\times10^{-5}$ ($0 - 1.67\times10^{-4}$) | 1 in 48,000 (0 - 1 in 12,000 ) |

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## Table 5.1B. Model Results for Total Population-based Exposure and Potential vCJD Risk for All Hemophilia A patients who use a Hypothetical pdFVIII Product with 4-6 $log_{10}$ Manufacture Process Reduction of vCJD Agent:

Predicted annual per person exposure to vCJD i.v. $ID_{50}$ and mean potential per person annual vCJD risk:

- For patients with SEVERE disease, and
- Two different UK vCJD prevalence estimates.

| | | | 4 - 6 $Log_{10}$ Reduction | | | |
|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani (2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | |
| | Est. Total Number severe HA patients in US | Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] |
| Mean total annual exposure and population risk | 1,800 | 243 million IU[d] | $6.50 \times 10^{-4}$ (0-0)[e] | 1 in 3,077 years (0-0)[e] | $5.67 \times 10^{-2}$ (0 - $2.52 \times 10^{-1}$) | 1 in 35 years (0 - 1 in 8) |

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.
[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity iv $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)
[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.
[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.
[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

## V. C. Model results: Estimated annual potential exposure to i.v. $ID_{50}$ vCJD agent and potential vCJD risk through human pdFVIII used to treat severe von Willebrand disease (vWD)

Individuals with von Willebrand disease (vWD) vary in severity of disease, those with Type 3 disease have severe disease; this assessment specifically addresses potential vCJD exposure and risk for persons with severe vWD. FDA estimates that approximately 250 vWD patients have severe vWD disease in the United States and use human plasma-derived FVIII products to control their disease (Tables 5.2A. and 5.2 B.) The FDA model suggests that it is possible that some of these vWD patients using human pdFVIII may potentially be exposed to vCJD agent if present in US manufactured product. Results from the risk assessment model for patients with vWD and treated with pdFVIII product with a 4-6 $\log_{10}$ manufacturing process reduction of vCJD agent are shown in Tables 5.2A. and 5.2 B. Generally results are expressed for patients with von Willebrand disease (vWD) clinical treatment groups of either Prophylaxis or Episodic treatment.

**Table 5.2A. Results von Willebrand Disease (vWD) patients[1] with Severe Disease: Predicted Potential Annual Exposure to vCJD i.v. $ID_{50}$ and vCJD Risk:**

- Assuming a processing reduction of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

| | | | 4 - 6 $\text{Log}_{10}$ Reduction | | | |
|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton, et al (2004) | |
| | Est. Total Number patients in US | Mean quantity product used per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] per person per year ($5^{th}$ - $95^{th}$ perc)[c] | Mean potential vCJD risk per person per year[b] ($5^{th}$ - $95^{th}$ perc)[c] |
| **YOUNG vWD (≤ 15 yrs of age)** | | | | | | |
| *Prophylaxis* | 39 | 165,713 IU[d] (9876, 454306) | $4.30\times10^{-7}$ (0 - 0)[e] | 1 In 4.7 million (0 - 0)[e] | $3.81\times10^{-5}$ (0 - $1.54\times10^{-4}$) | 1 in 52,000 (0 - 1 in 13,000) |
| *Episodic* | 60 | 11,045 IU[d] (1025, 34352) | $4.14\times10^{-8}$ (0 - 0)[e] | 1 In 48 million (0 - 0)[e] | $2.06\times10^{-6}$ (0 - $6.83\times10^{-5}$) | 1 in 971,000 (0 - 1 in 293,000) |
| **ADULT vWD (> 15 yrs of age)** | | | | | | |
| *Prophylaxis* | 73 | 186,880 IU[d] (16910, 539877) | $4.89\times10^{-7}$ (0 - 0)[e] | 1 In 4.1 million (0 - 0)[e] | $4.32\times10^{-5}$ (0 - $1.82\times10^{-4}$) | 1 in 46,300 (0 - 1 in 11,000) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Episodic* | 78 | 86,923 IU[d] (2182, 240338) | $1.99\times10^{-7}$ (0 - 0)[e] | 1 in 10 million (0 - 0)[e] | $1.90\times10^{-5}$ (0 - $8.43\times10^{-5}$) | 1 in 1 million (0 - 1 in 24,000) |

[1] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[a] iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[b] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity i.v. $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[c] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[d] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[e] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

*Estimation of Factor VIII product utilization by patients with severe von Willebrand disease.* FDA obtained data on pdFVIII utilization, presumably used in the treatment of severe von Willebrand disease, from the Centers for Disease Control (CDC). Details of the CDC – Six state collaborative study are described in the section above (section IV.G.2) on FVIII utilization. Annual usage of product by vWD patients was estimated based on an assumption that this patient class largely uses Humate P. Therefore, only records for patients utilizing Humate P were extracted from the CDC - Six state study conducted from 1993 – 1998 and used to develop statistical distributions of product usage for young vWD (≤15 yrs old) patients and adult vWD (> 15 yrs old) patients. The mean quantity of product utilized per year per patient group is shown in Table 5.2A. and Table 5.2B.

**Table 5.2B. Von Willebrand Disease (vWD) Patients[1] with Severe Disease: Predicted Total Population-based Exposure to vCJD i.v. $ID_{50}$ and Potential vCJD Risk:**

- Assuming a processing reduction of 4-6 $\log_{10}$, and
- Two different UK vCJD prevalence estimates.

| | | | 4 - 6 $\log_{10}$ Reduction | | | |
|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani (2005) | | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | |
| | Est. Total Number severe vWD patients in US | Mean Total quantity FVIII used by all patients per year (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] | Mean exposure to vCJD iv $ID_{50}$[a] of all patients per year (5th - 95th perc)[c] | Mean population – based potential vCJD risk[b] (5th - 95th perc)[c] |
| *Mean total annual exposure and population* | 250 | 29.9 million IU[d] | $7.05\times10^{-5}$ (0 - 0)[e] | 1 in 28,450 years (0 - 0)[e] | $4.91\times10^{-3}$ (0 - $2.59\times10^{-2}$) | 1 in 405 years (0 - 1 in 76) |

| | | | | | | |
|---|---|---|---|---|---|---|
| *risk* | | (3013, 311745) | | | | |

[a] Number (percent) patients in a CDC sponsored study with 6 states to survey treatment of Hemophilia A and B conducted 1993 - 1998. Our analysis included 14 patients (<15yrs) and 28 patients (≥15yrs) (total = 42) on prophylaxis or episodic treatment with Humate P only and no record of inhibitor.

[b] Iv $ID_{50}$ represents the probability that 50% of those exposed to 1 $ID_{50}$ intravenously may become infected with vCJD.

[c] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential annual vCJD risk = Total mean quantity i.v. $ID_{50}$ per year x 0.5 (50 % chance infection from $ID_{50}$)

[d] The 5th - 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[e] IU - represents international units of Factor VIII and may be expressed using the term "unit" or "units" in this document.

[f] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

*Potential exposure of severe von Willebrand disease patients to vCJD agent: Results based on lower epidemiological model estimated prevalence of ~1.8 in 1,000,000 (Clarke and Ghani, 2005).* Adult vWD (>15yrs of age) patients with severe disease on prophylaxis consumed the largest quantities of pdFVIII product annually and may potentially be at greater vCJD risk. Using the lower epidemiological model prevalence estimate, analysis of pdFVIII utilization data indicated that 73 Adult vWD patients on prophylaxis treatment regimen used an average of 186,880 IU and are potentially exposed to an average of 4.89 x $10^{-7}$ i.v. $ID_{50}$ per person per year, and representing an average potential vCJD risk of 1 in 4.1 million per person per year **(Table 5.2A.)**. At this level of risk, only 1 vCJD infection would be predicted to occur in an average of approximately 56,000 years. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~1.8 per million) in **Table 5.2A.** are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. Greater than 99% of the time (on average) the model estimates the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicts that 0.027% of the time the exposure to vCJD agent may be greater than zero, and there is a possible but low risk of vCJD infection.

Totaling the model results reveals that the approximately 250 severe vWD patients in the US used a total of 29.9 million IU, and are potentially exposed to an average total of 7.05 x $10^{-5}$ i.v. $ID_{50}$ per year. This represents an average potential vCJD risk of 1 in 28,450 **(Table 5.2B.)** or (as predicted by the model) roughly equal to one vCJD infection observed over a time span of approximately 28,450 years in the population of 250 severe vWD patients.

*Potential exposure of severe von Willebrand disease patients to vCJD agent: Results based on higher prevalence estimate of 1 in 4,225 (Hilton et al 2004).* At the higher surveillance prevalence estimate, among the vWD patient populations examined by the model, results **(Table 5.2A.)** indicated that adult vWD (>15yrs of age) patients with severe disease on prophylaxis used the largest quantities of pdFVIII product annually and may potentially be at greater vCJD risk. Analysis of pdFVIII utilization data indicated that 73 Adult vWD patients on prophylaxis treatment regimen used an average of 186,880 IU per person per year and are potentially exposed to an average of 4.32 x $10^{-5}$ i.v. $ID_{50}$ per person per year, representing an average potential vCJD risk of 1 in 46,300 per person per year **(Table 5.2A.)**. At this level of risk, only 1 vCJD infection would be predicted to occur in an

average of approximately 630 years for the population of 73 Adult vWD patients on prophylaxis treatment regimen.

The potential risk of vCJD infection for the entire population was calculated using the higher surveillance prevalence estimate. The model results shows that the approximately 250 severe vWD patients in the US used a total of 29.9 million IU (**Table 5.2B.**), and are potentially exposed to an average total of $4.91 \times 10^{-3}$ i.v. $ID_{50}$ per year. This represents an average potential vCJD risk of 1 in 405, i.e., of one vCJD infection observed over a time span of 405 years for the population of 250 severe vWD patients in the U.S.

*Range of Predicted annual mean potential per HA patient vCJD risk for pdFVIII (Table 6)*
The FDA risk assessment for potential vCJD infection risk for US manufactured pdFVIII generates results for several scenarios that reflect two key factors that greatly influence the final risk estimates including: (1) Reduction in vCJD agent in pdFVIII product during manufacture, and (2) UK vCJD prevalence estimate. As indicated earlier, the model used two widely different prevalence estimates, one lower prevalence estimate based on epidemiological modeling of predicted vCJD cases in the UK (Clarke and Ghani, 2005) of approximately 1.8 in 1 million and one higher prevalence estimate based on surveillance data of UK patient tissue samples (Hilton et al 2004) of 1 in 4,225. The use of these two estimates gives rise to a difference in results generated by the model that vary by an average of approximately 130 fold.

The model evaluated three separate categories of reduction in infectivity including 2-3 $\log_{10}$, 4-6 $\log_{10}$, and 7-9 $\log_{10}$. These three hypothetical categories were chosen to span the possible range of reduction of vCJD agent for pdFVIII products. **Table 5.3A. and 5.3B.** displays model results for a lower prevalence estimate and a higher prevalence estimate at all three levels of reduction. It should be noted that the mean difference between the lowest range of 2-3 $\log_{10}$ and the highest range of 7-9 $\log_{10}$ is nearly 1 million fold (6 $\log_{10}$). These two largest contributors to the final risk estimate also contribute to the greatest uncertainty in the model. Results from the model shown in **Tables 5.3A. and 5.3B.** indicate that there is a difference of approximately 20 to 55 million fold between the lowest and highest risk estimates of each patient group.

**Table 5.3A. Range of *Predicted Annual Mean Potential per HA Patient vCJD risk for pdFVIII* – at *three levels of clearance: 7-9 $log_{10}$, 4-6 $log_{10}$, and 2-3 $log_{10}$ and at a higher Prevalence and Lower Prevalence estimates and at .***

| | | | | 7 - 9 $Log_{10}$ Reduction | | 4 - 6 $Log_{10}$ Reduction | | 2 - 3 $Log_{10}$ Reduction | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) |
| Treatment Regimen | Inhibitor Status | Est. Total Number patients in US | Mean quantity product used per person per year ($5^{th}$ - $95^{th}$)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] |
| *Prophylaxis* | No Inhibitor | 578 | 157949 IU (21242, 382316) | 1 in 4.1 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 4 million (0-0)[c] | 1 in 54,000 (0- 1 in 12,000) | 1 in 15,000 (0-0)[c] | 1 in 82 (0 - 1 in 17) |
| | With Inhibitor – No Immune Tolerance | 63 | 190523 IU (26958, 447639) | 1 in 3.5 billion (0-0)[c] | 1 in 40 million (0 - 1 in 8.8 million) | 1 in 4.8 million (0-0)[c] | 1 in 41,000 (0- 1 in9,000) | 1 in 12,000 (0-0)[c] | 1 in 65 (0 - 1 in 13) |
| | With Inhibitor – With Immune Tolerance | 62 | 558700 IU (33235, 1592943) | 1 in 551 million (0-0)[c] | 1 in 15 million (0 - 1 in 3.4 million) | 1 in 1.3 million (0-0)[c] | 1 in 15,000 (0- 1 in3,700) | 1 in 2,700 (0-0)[c] | 1 in 24 (0 - 1 in 3 ) |
| *Episodic* | No Inhibitor | 946 | 85270 IU (4633, 244656) | 1 in 3.2 billion (0-0)[c] | 1 in 100 million (0 - 1 in 24 million) | 1 in 9.4 million (0-0)[c] | 1 in 105,000 (0- 1 in 24,000) | 1 in 21,500 (0-0)[c] | 1 in 159 (0 - 1 in 34 ) |
| | With Inhibitor | 151 | 180458 IU (5314, 488906 ) | 1 in 4 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 8 million (0-0)[c] | 1 in 23,000 (0- 1 in 12,000) | 1 in 23,000 (0-0)[c] | 1 in 73 (0 - 1 in 16) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range constituting the 90% confidence interval. Accordingly, the mean risk estimates from the model should fall within this defined interval at least 90% of the time.

[c] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.



BENE2006-025

This range or difference in the estimates of about 20 -55 million fold is reflected in the higher and lower prevalence results generated by the model shown in **Table 5.3A.** for each HA patient treatment group with severe disease. On closer inspection of the results in **Table 5.3A.** for patients with the most intensive pdFVIII product use, that is, the 62 patients on prophylaxis-with inhibitor and with immune tolerance, the effect of clearance on mean potential vCJD risk across the three ranges of clearance can be seen. At the low end of risk, the mean potential vCJD risk per patient per year risk (at 7-9 $\log_{10}$ and the lower prevalence estimate) is 1 in 551 million. Conversely, the highest risk for this patient group is seen at the 2-3 $\log_{10}$ clearance level and the higher prevalence estimate and is estimated by the model to be an average of 1 in 24. For patients on episodic treatment with no inhibitor who have a less intensive annual use of product, the model predicts the lowest risk (at 7-9 $\log_{10}$ and the lower prevalence estimate) to be 1 in 3.2 billion. The model predicts the highest risk for this group of patients, if they used pdFVIII product with a 2-3 $\log_{10}$ clearance level and the higher prevalence estimate, would be a mean potential per patient risk of 1 in 159.

**Table 5.3B. Range of Total Population-based Exposure and Potential vCJD Risk from Model** Predicted HA population with severe disease annual vCJD Exposure and Risk associated with use of plasma-derived Factor VIII:

- Lower Prevalence assumptions of Prevalence of 1.8 in 1,000,000 and 7-9 $\log_{10}$ reduction, and
- Higher Prevalence assumptions of Prevalence of 1 in 4,225 and 2-3 $\log_{10}$ reduction.

| | | | 7 - 9 $\log_{10}$ Reduction | | 4 - 6 $\log_{10}$ Reduction | | 2 - 3 $\log_{10}$ Reduction | |
|---|---|---|---|---|---|---|---|---|
| | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton et al (2004) | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani ( 2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) |
| | Est. Total Number severe vWD patients in US | Mean Total quantity FVIII used by all patients per year ($5^{th}$ - $95^{th}$ perc)[b] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] | Mean population – based potential vCJD risk[a] ($5^{th}$ - $95^{th}$ perc)[c] |
| ***Mean total annual exposure and population risk*** | 1,800 | 243 million IU | 1 vCJD infection in 1.8 million years (0-0)[c] | 1 vCJD infection in 35,000 years (0 -1 in 9,000) | 1 vCJD infection in 3,100 years (0 -0)[c] | 1 vCJD infection in 40 years (0 -1 in 10) | 1 vCJD infection in 8 years (0 -1 in 2) | ~13 vCJD infections per year (0- 54 vCJD infections) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[c] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

The results from the risk assessment model shown in **Table 5.3A.** show a wide range of difference in the predicted risk and displays the range in our uncertainty and knowledge in

predicting the potential vCJD infection risk for HA patients who use US manufactured human pdFVIII. However, as further scientific information and data become available in the future, the uncertainty in the model may decrease and the estimates of vCJD risk for recipients of pdFVIII may become more precise.

Evaluating the total vCJD infection risk for the severe HA population of 1,800 by summing the total annual exposure (at the higher vCJD Infection prevalence estimated), the model predicts that the population would use a total average of approximately 243 million IU FVIII. If the patient population used product that attained a clearance of 7-9 $\log_{10}$ and assuming the lower prevalence the model predicts that for the total patient population the mean total annual risk would be 1 case in 1.6 million years representing a negligible vCJD risk that would likely not give rise to new cases of the disease. At the other end of the spectrum at the 2-3 $\log_{10}$ clearance level and the higher prevalence the model predicts a mean of approximately 13 vCJD infections per year **(Table 5.3.B.)** for the patient population.

## V. D. Sensitivity analysis

Sensitivity analysis is used to identify the input parameter or parameters that have the greatest impact on the risk estimates generated by the model and are done by varying the values of key input parameters and evaluating the effect on the final risk estimate. Our goal in doing these analyses was to identify the key input parameters that have the greatest influence on annual exposure to the vCJD agent. The model was examined and candidate variables for the sensitivity analysis were chosen from the model that exhibited the largest potential for variability and/or uncertainty and those values are listed in Table 5-7. Importance analysis is a type of sensitivity analysis. Our importance analysis used two values, one at the minimum or 5$^{th}$ percentile value and one at the maximum or 95$^{th}$ percentile value to provide a reasonable estimate of impact across the range tested. The results from the importance analysis are displayed as tornado plots **(Figures 2.A., 2.B. and 2.C.)**, which graphically shows the relative influence of each input parameter evaluated on the final model estimates. The most influential factors are displayed at the top of the plot and those that are least influential or those with negative influence on the risk are at the bottom of the plot.

For the FVIII risk assessment the output being monitored in the sensitivity and importance analyses was annual exposure ($I_{yr}$) to vCJD agent quantified in i.v.$ID_{50}$ units. The sensitivity and importance analysis were conducted using the HA patient population on prophylaxis treatment regimens with inhibitor and being treated for immune tolerance as the example population used to do the analyses. This population displayed the largest mean usage and the widest range in product utilization. We assumed that the sensitivity and importance analysis results are representative of all the HA and vWD patient populations included in our study since all of the populations were assumed to differ only by the total average quantity of pdFVIII utilized per year.

The importance analysis was performed for each variable by doing two sets of simulations, each with 5,000 iterations. For each set of simulations the value of one testing variable was set at the minimum or 5$^{th}$ percentile value for the input distribution and the simulation run; for the second run the variable was set at the maximum or 95$^{th}$ percentile value and the simulation run. The importance analysis was run separately each time using one of the three surveillance estimate

ranges. The first analysis used a range of 0.7 to 700 per million, which encompasses the entire range for both the HIGH and LOW prevalence estimates. The second analyses used the higher vCJD Infection prevalence estimate of 1 in 4,225 (or 237 per million) derived from a tissue surveillance study (Hilton et al 2004). This prevalence was based on the variable ($P_{vCJD\text{-}Surv}$) in the model that used data from a tissue surveillance study. To do the sensitivity analysis we used a 5$^{th}$ percentile value of 49 per million and a 95$^{th}$ percentile value of 692 per million. The third set of analyses used the lower vCJD Case prevalence estimate of ~1.8 per million based on epidemiological modeling from actual vCJD occurrence conducted by Clarke and Ghani (2005). This prevalence was ($P_{vCJD\text{-}Epi}$) based on epidemiologic modeling and to do the sensitivity analysis we used a 5$^{th}$ percentile value of 0.7 per million and a 95$^{th}$ percentile value of 4 per million. The results of all simulations and the ranking of input parameters by their importance is represented graphically using a tornado plot shown in **Figures 2.A. , 2.B. and 2.C.** The tornado plot displays the correlations between key inputs in the model and the model output of exposure. A tornado plot prioritizes the various input factors with the most influential factors at the top and those that are least influential or those with negative influence on the risk are at the bottom of the plot.

**Table 5.4. Input Variables included in Importance Analysis**

| Description of variables | Name of input variable | Importance analysis values |
|---|---|---|
| Entire range of estimated vCJD prevalence in UK (cases/million) | $Prev_{vCJD\text{-}UK}$ | Minimum: 0.7<br>Maximum: 700 |
| High prevalence estimate of vCJD in UK (cases/million) | $Prev_{vCJD\text{-}UK(Surveillance)}$ | 5$^{th}$ perc: 49<br>95$^{th}$ perc: 692 |
| Low vCJD prevalence in UK (cases/million) | $Prev_{vCJD\text{-}UK\ (Epi\ model)}$ | 5$^{th}$ perc: 0.7<br>95$^{th}$ perc: 4.0 |
| Efficiency of donor deferral policy | $Eff_{Def}$ | Minimum: 85%<br>Maximum: 99% |
| Efficiency of i.c. versus i.v. route | $A_{ic\text{-}iv}$ | Minimum: 0.1<br>Maximum: 1 |
| Number of donors per plasma pool | $DR_{Pool}$ | Minimum: 6500<br>Maximum: 360000 |
| Quantity of i.c. infectivity in infected human blood | $I_{bl}$ | 5$^{th}$ perc: 2<br>95$^{th}$ perc: 30 |
| Manufacturing yield of FVIII (IU/L plasma) | $Y_{VIII}$ | Minimum: 120<br>Maximum: 250 |
| Log Manufacture Reduction of vCJD agent | $R_{Log}$ | Minimum: 2<br>Maximum: 9 |
| FVIII used per year (IU/year) | $IU_{yr}$ | 5$^{th}$ perc: 10000<br>95$^{th}$ perc: 4000000 |



552

Sensitivity analysis is used to study the quantitative relationship between the input variables and risk output. Same as in importance analysis, output to be monitored in sensitivity analysis is annual exposure ($I_{yr}$) to vCJD of young HA patients under prophylaxis treatment with inhibitor and immune tolerance treatment. Sensitivity analysis for an input variable consists of multiple simulations. In each simulation the testing input variable is fixed at one value within the input range. Results of sensitivity analysis are presented only for the most important input variables, which were identified by the ranking provided by the importance analysis.

**Fig 2. A. Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using prevalence estimate encompassing the range of values for both high and low prevalence from 0.7 to 700 vCJD cases per million UK population.** Tornado chart showing impact of input variables on estimated annual exposure of severe HA patient with prophylaxis, inhibitor and immune tolerance treatment

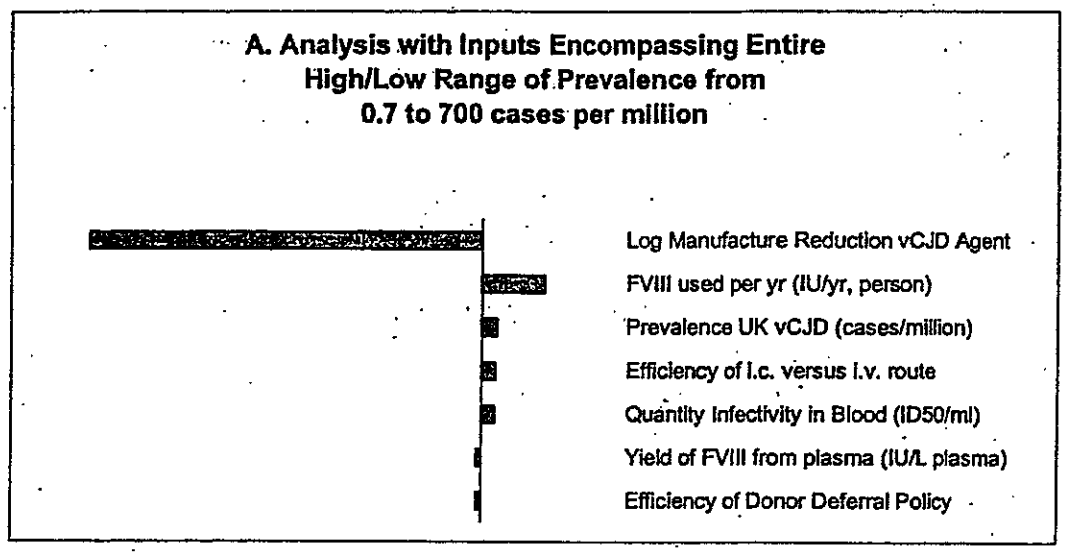



**Fig 2. B. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Tissue Surveillance-based (HIGH) prevalence estimate.** Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.

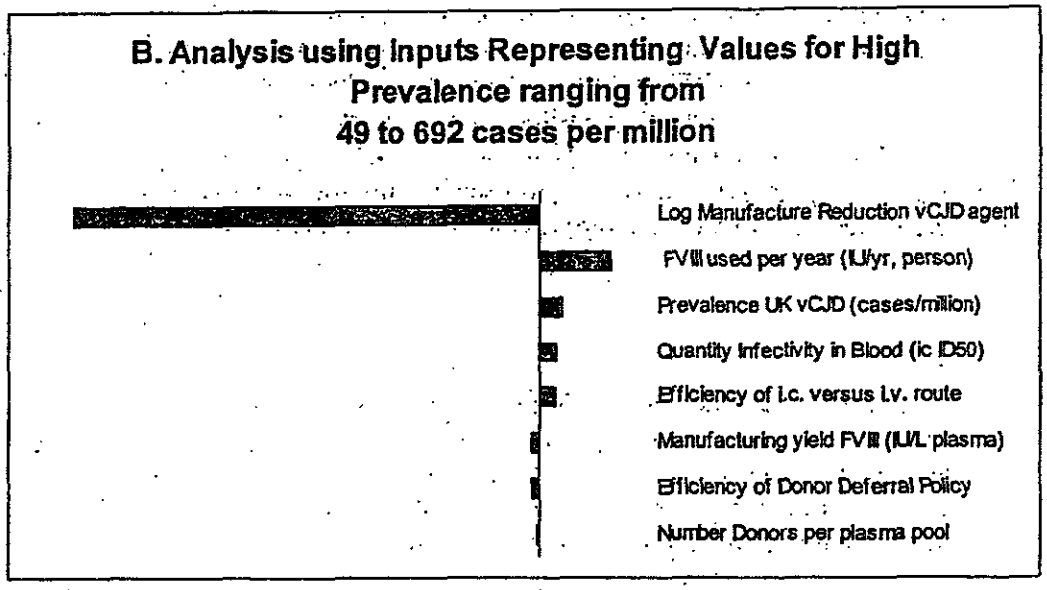
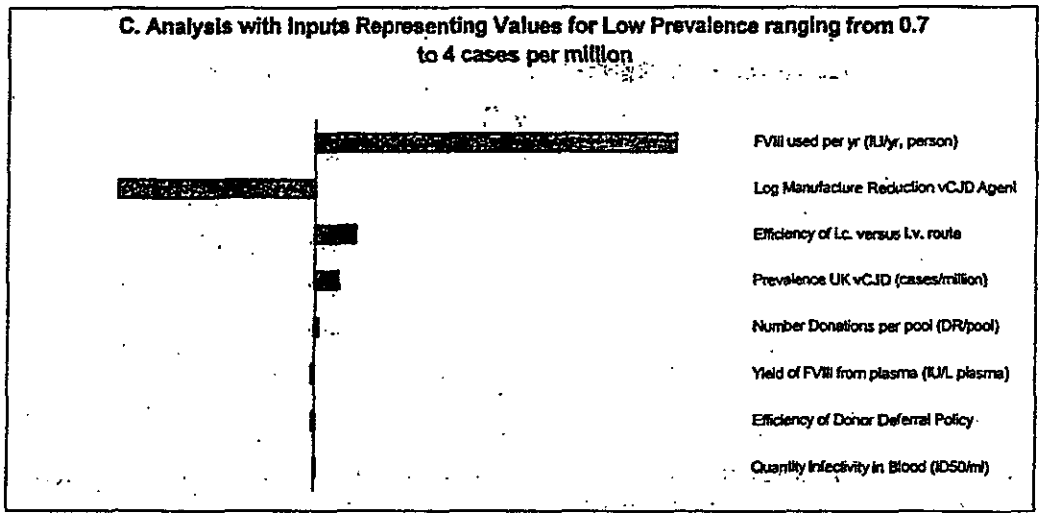



**Fig 2. C. FVIII Importance Analysis ranking influential factors for predicted annual vCJD exposure ($I_{yr}$) using Epi Modeling-based (LOW) prevalence estimate.** Tornado plot showing impact of input variables on estimated per treatment course exposure of pdFVIII recipients.




Some input variables are used multiple times in the original model, for instance each type of plasma pool (Source or recovered) was modeled on an individual basis. Other examples are pool size ($DR_{pool\text{-}S}$ and $DR_{pool\text{-}R}$), yield ($Y_{FVIII}$), quantity of i.c. infectivity in the infected human blood ($I_{bl}$) and the reduction of infectivity during manufacturing ($R_{Log}$). In importance analysis and sensitivity analysis, when these input variables are tested, we assumed that there was no difference among the pools. When evaluating the impact of a specific variable all other values are held constant during the simulation. When simulating parameters with multiple values (e.g., size of recovered plasma pools) all values are the same for the simulation. The magnitude of changes in risk output associated with changes of input variables are graphed in the tornado chart, which

represents the relative ranking of the input variables by their impacts on the risk outcome. The importance analysis was conducted for three possible ranges of UK vCJD prevalence: one set of analysis for tonsil survey based estimate, one set for epidemiology model-based estimate and another set for the two prevalence estimates combined.

The order of the influence of the specific input factors varies slightly when the importance analysis is conducted using the three difference prevalence estimates. When a higher prevalence estimate was used (either the combined prevalence (0.7 to 700 per million) the tornado plots in **Figures 2.A. and 2.B.** both show that clearance or Log reduction of the vCJD agent ($R_{Log}$) during the manufacturing process is the dominant factor that influences the annual exposure or risk for a pdFVIII recipient. The importance analysis suggests that changes in the input values for prevalence used in the analysis can cause some visible changes in the rank order of the influence of the various input factors. A change in the rank order of model factors is seen when the lower prevalence estimate of 0.7 to 4 per million is used (**Figure 2. C.**). The dominant factor potentially driving risk then becomes the quantity of pdFVIII used by a patient.

In our importance analysis the five variables that had significant influence on the output of the model were clearance of i.v. $ID_{50}$ from pdFVIII products, pdFVIII use (IU/yr), UK vCJD prevalence, adjustment for the efficiency of transmission via the i.c. route vs. the i.v. route, and the quantity of i.v.$ID_{50}$ in blood. Changes in prevalence did cause the variable parameters to reassort and change rank when the different prevalence estimates were used. Overall, however, they were somewhat similar in asserting their influence on the estimated risk outcome(s), but had significantly less influence when compared to that of reduction of infectivity during processing and manufacture. Although these types of sensitivity analysis and tornado plots are often used to identify influential factors of risk, their use has some limitations. Factors are examined singly or in isolation so interaction among various factors that may influence the risk estimate are not addressed.

### General comments on model outputs

The risk estimations in this section of the risk assessment are predicated on the assumption that there is homogeneous mixing and dispersion of vials from all pools among all donors. In reality, vials may not be dispensed homogeneously and it is likely that patients draw from only one or a few manufactured lots of pdFVIII product in a given year. FDA did not have data to model this non-homogeneous dispensing of pdFVIII but the model can be used to estimate the average maximal level of i.v. $ID_{50}$ exposure if on a very rare chance all vials used by a patient in a given year happened to contain vCJD agent.

## V. E. Uncertainty and Data Gaps

Uncertainty arises from the absence of information or availability of limited information. In our probabilistic model statistical distributions are used, where possible, to represent the uncertainty of much of the information used in the model. There are uncertainties in the information and the model that we were unable to quantify and that are not represented in the final risk estimates. Some of the difficult to quantify uncertainties are associated with the extrapolation of a human dose-response relationship based on animal data, an assumed linear dose response with no

uncertainty or variability bounds, and assumption of infectivity in the last 50% of the incubation period. We express the uncertainty of the final risk estimates generated from the model using a mathematical mean (average) of exposure in $ID_{50}$ units and the 5th and 95th percentiles, which represent the 90% confidence interval for each estimate. The uncertainty for the risk estimates generated by this FVIII risk assessment model is significant and decision makers should use the results with caution. Similarly, patients and physicians should understand that the uncertainties are too great at this time to determine the presence, absence or degree of actual risk. In the future, additional research and information may be substituted for assumptions or used to improve estimates for the individual parameters and ultimately improve the precision of the final risk estimates generated by the model.

Even considering the associated uncertainty of estimated risks, risk assessment provides an estimate of risk based on the current and known information. It is still a useful tool that can inform the science-based decision making process. It can identify data gaps and research priorities where additional research and information would have the greatest impact on enhancing the final risk estimates. The sensitivity analysis results in Section IV.C. indicated that the risk assessment results are highly dependent upon log reduction of vCJD agent ($R_{Log}$) during the manufacturing process. The modeled estimates were based upon levels of reduction seen for manufacturing steps of several different types of plasma-derived products that were similar in some but not all respects to those used in the manufacture of FVIII products. More high quality data on the levels of vCJD agent clearance achieved during the pdFVIII manufacturing would likely improve the final risk estimate generated by the FDA model. Given the lack of data on vCJD agent clearance for pdFVIII uncertainty is considerable.

Better information on when infectivity is present in human blood during the incubation period is a critical factor in the model, especially if the higher vCJD infection prevalence estimate (of 1 in 4,225) is in the range of the actual vCJD prevalence, and would improve predictions generated by the model. There are no data available on the level of infectious units or $ID_{50}$ units present in the bloodstream of vCJD infected individuals at the time of blood donation. The model extrapolates an estimate of the level of vCJD agent that might be present in human blood based on data from several animal models. However, the presence and level of agent present in an infected individual at the time of blood donation could differ from our assumption and this adds to the uncertainty of the risk assessment outcomes.

The model estimates exposure to the vCJD agent in the form of intravenous $ID_{50}$ units. Data are not available to estimate the probability of various clinical outcomes, such as infection or illness that might be predicted to arise from exposure to a particular level of agent. Although we did estimate a probability of infection in our model, the uncertainty associated with the estimate is considerable. However, a meaningful dose-response model would need to be generated for vCJD exposure in humans to improve estimates of the probability of adverse clinical outcomes for humans. The type of data needed to generate a dose-response model that would improve the quality of TSE risk assessment predictions would necessitate injection of groups of animals at several different concentrations of $ID_{50}$, including low doses below 1 $ID_{50}$ using a protocol that mimics transfusion transmission of vCJD in humans. Both infection and duration of the incubation periods at several different i.v. $ID_{50}$ concentrations would be useful endpoints for developing informative dose-response relationships. Given the state of the current TSE science, estimates of the probability of vCJD infection or illness arising from exposure to the vCJD agent are still extremely uncertain. Nevertheless risk assessment is a tool that provides insight into

important factors where additional research is needed into production processes, tools, or strategies that may further reduce vCJD risks and advance product safety for patients.

The manufacturing processes for pdFVIII are highly varied – therefore, any potential clearance of the vCJD agent during production is likely variable and dependent upon the specific steps used to produce the final product. For example, the techniques applied in fractionation process vary from manufacture to manufacture including the sizes of plasma pools used for producing pdFVIII, the yield of products, and the reduction of infectivity during processing varies within a limited range from batch to batch. In addition the utilization of pdFVIII varies from individual to individual. This risk assessment considers the typical production and utilization. Uncertainty from the model should be appreciated. Human plasma-derived FVIII is typically prepared through successive steps of large scale fractionation during the manufacturing process. Cryoprecipitation is the first and a common step in preparation of pdFVIII. Afterward, cryoprecipitate undergoes further fractionation procedures such as precipitation, absorption/desorption, ion exchange and filtration to yield intermediate purity FVIII, [redacted] In certain cases some hospitals may prepare small amount of cryoprecipitate FVIII from small plasma pools (1-8 donations/pool) for special treatment purposes. Preliminary risk assessment results indicated that the risk that vCJD would be transmitted through cryoprecipitated AHF is relatively low due to the small size of plasma pool and small numbers of donors involved. This risk assessment uses 3 ranges of possible clearance of vCJD agent from pdFVIII of 2-3 $\log_{10}$, 4-6 $\log_{10}$, and 7-9 $\log_{10}$ to cover the possible ranges for all pdFVIII products presently in the marketplace.

## General comments on model outputs

The risk estimations in this section of the risk assessment are predicated on the assumption that there is homogeneous mixing and dispersion of vials from all pools among all donors. In reality, vials may not be dispensed homogeneously and it is likely that patients draw from only one or a few manufactured lots of pdFVIII product in a given year. FDA did not have data to model this non-homogeneous dispensing of pdFVIII but the model can be used to estimate the average maximal level of i.v. $ID_{50}$ exposure if on a very rare chance all vials used by a patient in a given year happened to contain vCJD agent.

## V. F. Conclusions

Results from the FDA pdFVIII risk assessment model suggest that the risk of vCJD infection from US manufactured pdFVIII generally appears likely to be very low, but may not be zero. For US plasma donors, the major source of vCJD risk is dietary exposure during travel and/or residency in the UK, France, or other countries in Europe since 1980. Although donor deferral criteria in place since 1999 have reduced the risk of donation by exposed persons some are not deferred and potentially may donate plasma that contains the vCJD agent. However, the model suggests that the likelihood of a vCJD contaminated plasma pool is low.

Manufacturing processes for human pdFVIII products likely reduce the quantity of vCJD agent, if present, but the level of reduction through manufacturing steps is not precisely known. Clearance of TSE agents in manufacturing appears to vary among products, but has not been measured in standardized studies which might allow more meaningful direct comparisons. Based on currently available experimental studies, it is estimated that pdFVIII products potentially have

4 $\log_{10}$ (or 10,000 fold) or greater manufacturing process reduction of the vCJD agent. Assuming a 4-6 $\log_{10}$ manufacturing process reduction, the modeling predicts that the potential risk per person per year for patients with severe HA using pdFVIII ranges from1 in 15,000 for the higher vCJD prevalence estimate and high product usage to 1 in 9.4 million for the lower vCJD prevalence estimate and low product usage. Due to the wide range of methods used for clearance studies currently available, gaps in information, and the results of the model, it is not possible at this time to determine with any certainty if a specific product may be less or more safe than another.

Although results of the model suggest exposure to vCJD agent is possible, and there is a potential risk of infection that is likely to be very low, it is not possible for the model to provide a *precise* estimate of the vCJD risk in general, or of the actual risk to individual patients. Although the actual risk is highly uncertain, the risk assessment model indicates that the most important factors affecting risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, and the vCJD prevalence in the UK donor population.

*In considering the results of the risk assessment it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products, including pdFVIII.. This includes patients receiving large amounts of other products manufactured from UK plasma donations over a long period of time.* This observation suggests that the actual risk of vCJD infection from pdFVIII is likely to be very low. The absence of cases does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

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# APPENDIX A

## Supplemental technical information for the FDA Risk Assessment

The sections in this appendix provide additional technical information and details of data and modeling approaches used in specific sections (but not all sections) of Section IV. The heading and numbering of each section in this appendix mirrors the sections in "Section IV. Exposure Assessment" portion of the risk assessment.

### A- IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

### A-IV. A. 3. Estimation of age-specific vCJD prevalence based on the age distribution of diagnosed vCJD cases in the UK

Cases of vCJD occur in relatively young individuals (median age of 28 years) compared to classic CJD. Blood and plasma donors are usually at the age 18-40, among whom the vCJD prevalence would be expected to be higher than the prevalence among general population. Because age specific rates of donation and vCJD infection would likely have a large effect on the final risk estimate the FDA model carefully characterizes the age specific prevalence of vCJD and donation rate. Throughout the FDA model, age specific vCJD prevalence rates are calculated for each five year age group beginning at age group of 10 – 14 yrs, 15-19 yrs and so on – and applied in estimating vCJD risk and prevalence for the residents of different geographic regions and the US blood and plasma donors who traveled to those regions. The percentage of reported vCJD cases by age is shown in **Table A-4.3.** The model assumes that the age-specific percentage and prevalence of incubating asymptomatic cases reflects the same age-specific trend as for reported cases of symptomatic vCJD and deaths from vCJD.

**Table A-4.3. Reported vCJD cases in the UK and percent of US Source Plasma and blood (recovered plasma) donors by age groups**

| Age group | <10 | 10-14 | 15-19 | 18-19 | 20-24 | 25-29 | 30-34 | 35-39 | 40-44 | 45-49 | 50-54 | 55-59 | 60-64 | 65-69 | >70 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Reported vCJD cases in UK (through 2003)[a] (%)** | 0 | 5 (3.4%) | 27 (18.4%) | | 32 (21.8%) | 30 (20.4%) | 22 (14.9%) | 13 (8.8%) | 5 (3.4%) | 3 (2%) | 5 (3.4%) | 0 (0%) | 5 (3.4%) | | |
| **Age distribution of US Source Plasma donors (%)[b]** | 0 | 0 | 0 | 12% | 29.3% | 14.1% | 14.1% | 9.6% | 9.6% | 5.8% | 5.8% | 0% | 0% | 0% | 0% |
| **Age distribution of US Blood (Recovered plasma) donors[c]** | 0 | 0 | 0 | 5% | 13% | 8% | 10% | 12% | 13% | 12% | 11% | 7% | 4% | 5% | 0% |

[a]Hilton *et al.* 2004

[b]Plasma Protein Therapeutics Association (Jan 07, 2005). Where data were organized in broader age group we allocated donor equally among smaller 5 year age groups

[c] Data provided to FDA by Westat in 2002

Some of the general variables for generating age specific estimates from the model are described below.

**Variable:** ***age*** - Age of vCJD cases in 5-year increments

**Variable:** $vCJD_{UK(age)}$ - Reported vCJD cases in the UK by 5-year age groups (through 2003) beginning at 10 – 14 yrs, 15-19 yrs and so on.

**Data used in the model:** Data on the vCJD cases in the UK was derived from Hilton et al. (2004). The data includes cases through the end of 2003.

**Variable:** $Perc_{vCJD(age)}$-Percentage vCJD cases attributed by each age group from 10 – 14 yrs, 15-19 yrs and so on.

**Assumption used in the model:** We assume each of four age groups, 55-59 yrs, 60-64 yrs, 65-69 yrs and 70-74 yrs, contributes same percentage in vCJD cases.

For the four (five-year) age groups from 55-74yrs we assumed an equal percentage of cases were present in each of the four groups since there were very few vCJD cases in this age range. To estimate the percentages for each group we identified five reported cases (specifically, three reported cases in the age-specific prevalence grouping shown in Hilton et al. (2004) for persons aged 55-74 yrs and two cases of blood transfusion vCJD (each > 64 yrs of age) (Llewelyn 2004, Peden 2005)) in the 55-74 yr age range. The five cases in the 55-74 yr old age group are shown in **Table A-4.3.** We assumed an average of 1.25 cases for each of the four age groups from the ages of 55-74 yrs and divided by the total number of vCJD cases for all age groups to get the percentage of cases for each of the four sub age groups, 55-59 yrs, 60-64 yrs, 65-69 yrs and 70-74 yrs.

The percentage of vCJD cases in the UK from each age group is represented by the equation:

$$Perc_{vCJD(age)} = (vCJD_{UK(age)} / \sum_{age=0-4}^{\geq 85} vCJD_{UK(age)}) \times 100\% \qquad \text{(IV. A. 1-1)}$$

## A-IV. A. 3. a. Estimating the UK vCJD prevalence predicted by epidemiological modeling (Clarke and Ghani 2005) for each age group

**Variable:** $vCJD_{since2002}$-Predicted clinical vCJD cases in the UK from 2002 afterward (cases). Predicted clinical cases from 2002 afterward include 32 vCJD cases diagnosed in 2002 and 2003 and 70 (95% CI of 10-190) future cases between 2004 and 2080 predicted by Clarke and Ghani (2005), which give a total of 102 cases (95% CI: 42-222 cases) after 2002.

**Variable:** $Asym\text{-}vCJD_{(age)}$ - Number of asymptomatic vCJD infected individuals from a specific age group in year 2002.

$$Asym\text{-}vCJD_{(age)} = vCJD_{since2002} \times Perc_{vCJD(age)} \qquad \text{(IV.A.2-4)}$$

APPENDIX A

**Variable:** $Prev_{Asym\text{-}vCJD(age)}$- Prevalence of asymptomatic vCJD infected individuals in the UK by age groups (cases/million).

The prevalence of asymptomatic vCJD cases in the UK by age group is estimated using the equation:

$$Prev_{Asym\text{-}vCJD(age)} = Asym\text{-}_{vCJD(age)} / Pop_{UK(age)} \qquad \text{(IV.A.2-5)}$$

## A-IV. A. 3. b. Estimating the UK vCJD prevalence derived from tissue surveillance for each age group

For the risk assessment model we converted the 3 in 12,674 presumptive positive rate to an average rate of vCJD in the UK population of 1 in 4,225 (and used the 1 / 20,300 to 1 / 1,450 at 95% CI; proportions were converted from the 95% CI reported by Hilton et al (2004)). Demographic information of reported vCJD cases **(Table A-4.3)** indicated that the younger population (20 -29 yrs of age) that was deliberately oversampled in this study may have been more susceptible to the disease. The vCJD prevalence among UK donors might, therefore, be over-represented by the prevalence of 20-29 years age group derived from the surveillance study. Assuming the sensitivity and specificity of the testing method is 100%, the estimated rate of 1 in 4,225 translates roughly to a vCJD prevalence of 237 cases per million (95% CI: 49 – 692 cases per million) for all age groups. The authors (Hilton et al 2005) indicated that approximately 60% of the samples tested (from 7,600 patients) came from patients 20-29 years of age. Among the 20-29 year old group we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million.

We then derived the prevalences for the remainder of the UK donor population by determining the proportional difference between the vCJD prevalence from the tissue study group and the number of actual reported vCJD cases for donors in the 20-29 years age group. This proportion was then applied to the remaining age groups in the distribution of reported vCJD cases to determine the prevalence for each age group. By multiplying our extrapolated vCJD prevalence for incubating cases by the total donor population we were able to estimate the number of possible incubating vCJD cases in each US donor age group. We assumed that a plasma pool used to manufacture pdFVIII product in the US in the year 2002 consisted 6,000 to 360,000 donations, and several donations in the pool likely came from the same donor. The estimated prevalence was then used to generate variables and parameters representing the potential number of vCJD donors or donations that might be present in a plasma pool.

**Variable:** $Prev_{Asym\text{-}vCJD(20\text{-}30)}$. Prevalence of asymptomatic vCJD infected individuals in the UK 20-30 year old age group (cases/million)

**Assumptions used in the model:** The vCJD infectious agent is present in the blood of the individual when the the accumulation of prion protein can be detected in lymphoreticular tissue. Prevalence of vCJD asymptomatic individuals in the UK 20-30 year old age group is likely to be 400 cases/million, 95% CI=100-1200 cases/million. The values for this variable were estimated from the Hilton *et al* studies (2000, 2002, 2004).

**Variable:** $Pop_{UK(age)}$- Population in the UK by age groups (Thousands).

**Data used in the model:** The data for UK population were sourced from UK government statistics (UK National Statistics, 2005). Where UK data were organized in broader categories of 10 to 15 years we allocated population equally among smaller 5 year age groups.

**Variable:** $Asym\text{-}vCJD_{(20\text{-}30)}$ - The number of asymptomatic vCJD infected individuals in the 20-30 yr-old UK age group. This variable is represented by the equation:

$$Asym\text{-}vCJD_{(20\text{-}30)} = Prev_{vCJD(20\text{-}30)} \times Pop_{UK(20\text{-}30)} \quad \text{(IV.A.2-1)}$$

**Variable:** $Asym\text{-}vCJD_{(age)}$ - Number of asymptomatic vCJD infected individuals in the UK by age groups

**Assumptions used in the model:** Number of asymptomatic vCJD infected individuals from an age group is proportional to the percentage of reported vCJD cases from that age group. The age distribution of asymptomatic vCJD cases was assumed to be the same as that of symptomatic cases.

The number of asymptomatic vCJD individuals in the UK per age group was estimated using the following equation:

$$Asym\text{-}vCJD_{(age)} = Asym\text{-}vCJD_{(20\text{-}30)} \times (Perc_{vCJD(age)} / Perc_{vCJD(20\text{-}30)}) \quad \text{(IV.A.2-2)}$$

**Variable:** $Prev_{Asym\text{-}vCJD(age)}$- Prevalence of asymptomatic vCJD infected individuals in the UK by age groups (cases/million).

The prevalence of asymptomatic vCJD cases in the UK by age group is estimated by the equation:

$$Prev_{Asym\text{-}vCJD(age)} = Asym\text{-}_{vCJD(age)} / Pop_{UK(age)} \quad \text{(IV.A.2-3)}$$

## A-IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools (Module 2)

### A-IV. B. 1. a. Annual US plasma donors and characterization by age

### A-IV. B. 1. b. Source Plasma collection in the United States: characterized by donor age

**Variable:** $DN_S$– Annual number of Source Plasma units used to make pdFVIII .

**Assumption used in the model:** It was assumed that, on average, 3.3 million units of Source Plasma were used in each year to make pdVIII. It was further assumed that there is a 10% standard deviation in the number of Source Plasma units used to make pdFVIII for any given year.

**Data used in the model:** The annual number of Source Plasma units was back calculated based on annual units of pdVIII product made from Source Plasma, the average yield of pdFVIII (187 units per liter

plasma) and average volume of single unit of Source Plasma (700 ml per unit). The information on annual units of pdFVIII made from plasma collected in the US, yield of factor VIII and unit volume of plasma were collected from pdFVIII manufacturers.

**Variable:** $DR_S$–Annual number of donors who contribute Source Plasma for manufacture of pdFVIII.

**Assumption used in the model:** It was assumed that there are approximately 1 million Source Plasma donors in the US each year. It was further assumed that Source Plasma from any individual donor may be used to make pdFVIII. Therefore, we calculated that there were approximately 1 million donors who contributed Source Plasma for the manufacture of pdFVIII. It was further assumed that there could be a 10% standard deviation in the number of donors in any given year.

**Variable:** *Age* – Age information for US plasma donors was grouped in a two year increment for 18-19 years old because the model assumed that 18 was the minimum age of donation. The remaining population was grouped by 5-year increments – including 20- 24yrs old, 25-29yrs old, and so on

**Variable:** $DR_{S\text{-}perc(age)}$ - The percentage of Source Plasma donors from a given age group.

**Data used in the model:** Distribution of US Source Plasma donors by age was obtained from the Plasma Protein Therapeutics Association (2005). Where data (PPTA, 2005) were organized in broader age groups of 10 years or 15 years, we generated 5-year age subgroups by allocating the percentage equally among each subgroup.

**Variable:** $DR_{S(age)}$ – The annual Source Plasma donors by age groups who contribute plasma for pdFVIII manufacturing is represented by the equation:

$$DR_{S(age)} = DR_S \times DR_{S-perc(age)} \qquad \text{(IV.B.1-1)}$$

**A-IV. B. 1. c. Recovered plasma collection in the United States: Characterized by donor age**

**Variable:** $DN_R$ - Annual units of recovered plasma used to make pdFVIII.

**Assumption used in the model:** It was assumed that approximately 1,800,000 units of recovered plasma are used to make pdFVIII annually. This estimation was generated by backcalculation beginning with the total quantity of pdFVIII manufactured in the US. It was further assumed that there was a 10% standard deviation in the number of units for any given year.

**Data used in the model:** The annual number of total units of pdFVIII manufactured from recovered plasma collected in the US was estimated by back calculation. The calculation was based on the total quantity of annual units of pdVIII product made from recovered plasma collected in the US. We can further estimate the number of donations used to make the pdFVIII from recovered plasma using estimates in the literature for the average yield of pdFVIII 187 units per liter of plasma (WFH, 2004) and average volume of single unit of recovered plasma (200 ml per unit). The information on annual units of pdFVIII made from plasma collected in the US was collected from pdVIII manufacturers.

**Variable:** $DN_{Bl\text{-}perc(age)}$ – The percentage of blood units donated by a given age group.

Data used in the model: Distribution of blood units by donor age group was obtained from Westat data provided to FDA in 2002 (Data shown in A-4.3).

**Variable:** $DN_{R(age)}$ – Annual units of recovered plasma used to make pdFVIII by donor age group

$$DN_{R(age)} = DN_R \times DN_{Bl-perc(age)} \quad \text{(IV.B.2-1)}$$

**Variable:** $DR_{R(age)}$ – Annual number of donors by age group who contribute recovered plasma that is used for manufacture of pdFVIII

Assumption used in the model: Each unit of recovered plasma used to make pdFVIII comes from different donors. Therefore, number of donors from an age group equals the number of donations from that age group.

The annual number of recovered plasma donors by age group was calculated using the equation:

$$DR_{R(age)} = DN_{R(age)} \quad \text{(IV.B.2-2)}$$

**Variable:** $DR_R$ - The annual total of potential recovered plasma donors who contribute the plasma that is used for manufacture of pdFVIII, which was estimated in the model using the summation function:

$$DR_R = \sum_{age=12-19}^{70-74} DR_{R(age)} \quad \text{(IV.B.2-3)}$$

## A-IV. B. 2. Total plasma donors and donations- for manufacture of pdFVIII in the US

**Variable:** $DR_{Tot}$ - The annual total of potential plasma donors who contribute plasma for pdFVIII manufacturing is estimated by summing the number of Source Plasma donors and recovered plasma donors and is represented by the equation:

$$DR_{Tot} = DR_S + DR_R \quad \text{(IV.B.3-1)}$$

**Variable:** $DN_{Tot}$ - The annual total of potential plasma units used to make pdFVIII is estimated by summing the number of Source Plasma donations and recovered plasma donations and is represented by the equation:

$$DN_{Tot} = DN_S + DN_R \quad \text{(IV.B.3-2)}$$

## A-IV. C: Estimation of the probability that a plasma pool may contain a donation from an infected donor that contains vCJD agent

## IV.C. 1. US plasma donors with history of travel to the UK, France or other Countries in Europe: Annual number potentially infected and vCJD agent is present in the blood

## A-IV. C. 1. a. US plasma donors with history of travel to the UK: Number of donors potentially infected and vCJD agent is present in the blood

## A-IV. C. 1. a. i. US plasma donors with history of travel to the UK: Percentage of donors and duration of travel

The risk of vCJD infection in US plasma donors is a function of the intensity of exposure to the BSE agent. The intensity of exposure is assumed to be proportional to the amount of time spent, or duration of travel, in the UK and the prevalence of BSE in UK cattle during the period from 1980 – 1996. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 3 months) in the UK during 1980-1996, and to derive the frequencies for various durations of travel for 3 months or more. The period of 3 months or more corresponds to the length of time in the current policy that defers donors that traveled to or resided in the UK. The travel survey data on blood donors pose a limitation because the survey was conducted on whole blood donors and may not exactly reflect the travel histories of plasma donors. Unfortunately, to our knowledge there is not travel data available on plasma donors. Therefore, we assumed that plasma donor travel characteristics to the UK and other countries in Europe since 1980 are similar to those of whole blood donors and used this information in the FDA risk assessment. Some may argue that plasma donors travel less frequently than their blood donor counterparts so use of data on blood donors may overestimate the risk.

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC 2000).

**Variable:** $i$ - The duration interval used to group donors who traveled to the UK from 1980-1996 based on the quantity of time spent in the UK during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (in months) for interval $i$ representing the duration of travel or residence by US donors in the UK during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}UKi}$ – The cumulative percentage of blood donors who traveled to the UK within duration interval $i$ or longer.

**Variable:** $Perc_{BIDR\text{-}UKi}$ - Percentage of blood donors who traveled to the UK within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}UKi}$

**Variable:** $Perc_{BIDR\text{-}UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval *i* among all donors who have ever traveled to the UK is represented by the equation:

$$Perc_{BIDR-UKi/UK} = (Perc_{BIDR-UKi} / CumPerc_{BIDR-UK,i>1day-1month}) \times 100\% \quad \text{(IV.C.1.a-1)}$$

## A-IV.C.1.a.ii. All US plasma donors with a history of travel to the UK: Percentage and number of donors in each age group by year and duration of travel

For the purposes of our analyses we grouped all donors and donors who traveled to the UK between 1980 and 1996 into age groups of five year increments (20 – 24yrs, 25 – 29 yrs, etc). Because the minimum age of donation is 18, the model also included the donor group 18 & 19 years of age. The percentage of donors in each age group who traveled to the UK between 1980 and 1996 was calculated based on the total annual number donors who traveled to the UK between 1980 and 1996 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel.

As mentioned earlier, data are lacking on the travel characteristics of plasma donors, so the FDA used travel survey data collected from blood donors to estimate past travel history. Because plasma donors are less likely to travel use of these data may yield an overestimate of the actual risk. Characteristics of blood donors on travel including the percentage of donors from each age group who traveled to the UK during period between 1980 and 1996, and distribution of donor travel by duration were applied to plasma donors for estimation of the number of plasma donors from each age group who have traveled or resided in the UK from 1980 to 1996 for specific periods of time ranging from less than 3 months to greater than 5 years duration. Furthermore, the model used data that detailed the number of annual visits of US travelers to the UK to allocate donor travel specifically to an individual calendar year.

- **Calculation of the annual number of blood donors who traveled to the UK from 1980 through 1996**

Blood donors donate whole blood and soon after the liquid plasma portion of whole blood is separated and plasma is called recovered plasma. The model assumes that approximately 200 mls of recovered plasma are produced from a unit of whole blood.

**Variable :** $DR_{BI}$-The annual total number of potential blood donors in the US per year

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR\text{-}UK}$ - Percentage of US blood donors who traveled to the UK during the period from 1980 through 1996.

**Data used in the model:** Approximately, 22.5% of US blood donors have a history of travel to the UK any time during the period from 1980 through 1996, according to data contained in the National Blood Donor Travel Survey (TSEAC 2000).

**Variable:** $DR_{BI\text{-}UK}$ -Total number of blood donors estimated to have traveled to the UK from 1980 through 1996

$$DR_{BI-UK} = DR_{BI} \times Perc_{BIDR-UK} \quad \text{(IV.C.1.a-2)}$$

- **Calculation of the percentage of blood donors for each specific age group**

**Variable:** *Age*- Age of donors grouped in 5 year increments (e.g., 20 – 24 yrs, 25 – 29yrs, etc.) per year and 18-19 yr old age cohort.

**Variable:** $Perc_{BIDR(age)}$ Percentage blood donors in a specific age group.

**Variable:** ***BLDR(age)***- Annual number of blood donors in a specific age group

$$Perc_{BLDR(age)} = BLDR(age) / \sum_{age=18-19}^{65-69} BLDR(age) \quad \text{(IV.C.1.a-3)}$$

**Variable:** ***BLDN***-Annual total number of blood donations in the US.

**Variable:** $Perc_{BIDN(age)}$- Percentage of blood donations in each specific age group of US donors.

**Data used in the model:** Percentage of blood donations by age group was obtained from Westat (2002).

**Variable:** $Freq_{BIDN(age)}$-Average annual number of blood donations from a donor in a specific age group

**Data used in the model:** Information on the average annual number of donations bydonors in each specific age group was obtained from Westat (2002).

$$BLDR(age) = BLDN \times Perc_{BLDN(age)} / Freq_{DN(age)} \quad \text{(IV.C.1.a-4)}$$

Replace *BLDR(age)* in equation IV.C.1.a-3 with equation IV.C.1.a.-4, resulting in the expression:

$$Perc_{BIDR(age)} = (Perc_{BIDN(age)} / Freq_{BIDN(age)}) / \sum_{age=18-19}^{65-69} (Perc_{BIDN(age)} / Freq_{BIDN(age)}) \quad \text{(IV.C.1.a-5)}$$

**Variable :** $DR_{BI}$-The annual total number of blood donors in the US, which is assumed to be 8 million.

**Variable:** $DR_{BI(age)}$- The annual number of blood donors in each five-year age group and the18-19 yr old age cohort.

$$DR_{BI(age)} = DR_{BI} \times Perc_{BIDR(age)} \quad \text{(IV.C.1.a-6)}$$

**Variable:** $DR_{BI\text{-}UK}$ -Total number of US blood donors who have traveled to the UK during the period 1980 - 1996

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ - The percentage of US blood donors in an age group who traveled to the UK during the period 1980 - 1996.

$$DR_{BI-UK} = \sum_{age=18-19}^{65-69} (Perc_{BIDR-UK(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.a-7)}$$

**Variable:** $Odd_{T(age)}$- Age specific odd ratios for travel compared to the age group 18-19 years.

**Data used in the model:** The odds ratios for likelihood of travel for each age group were derived from the travel data obtained from 1980-1996 blood donor travel survey. An odds ratio of 1 was assigned to the donor group aged 18-19 years. The odds ratios for other age groups is a function of the travel frequency of those age groups compared to the travel frequency of the age group of 18-19 years

$$Perc_{BIDR-UK(age)} = Odd_{T(age)} \times Perc_{BIDR-UK(18-19)} \qquad \text{(IV.C.1.a-8)}$$

Replacing the variable, $Perc_{BIDR\text{-}UK(age)}$, in equation IV.C.1.a-7 with equation IV.C.1.a-8 the percentage of blood donors in the age group of 18-19 years of age who traveled to the UK was calculated using the following equation:

$$Perc_{BIDR-UK(18-19)} = DR_{BI-UK} / \sum_{age=18-19}^{65-69} (Odd_{T(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.a-9)}$$

## A-IV. C. 1. a. ii. a. Number of US Source Plasma donors who traveled to the UK in a specific year from 1980 to 1996 and by age group

**Variable:** *age* - Age of US plasma donors in groups by 5-year increments and 18-19 yr old age cohort.

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B. 1.) - The annual number of donors by age group who contribute Source Plasma to make pdFVIII.

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ (calculated in section A-IV.C.1.a.i.b.) - the percentage of blood donors in each age group who traveled to the UK between 1980-1996.

**Variable:** $DR_{S\text{-}UK(age)}$ - Number of Source Plasma donors who traveled to the UK from 1980 through 1996 by age groups.

**Assumptions used in the model:** The percentage of Source Plasma donors who traveled to the UK is the same as the percentage of blood donors of the same age group.

The number of Source Plasma donors who traveled to the UK from 1980 to 1996 by age group is represented by:

$$DR_{S-UK(age)} = DR_{S(age)} \times Perc_{BIDR-UK} \quad \text{(IV.C.1.a-8)}$$

The risk of vCJD infection in US donors who traveled to the UK was assumed proportional to the relative level of exposure to the BSE agent in each year through the UK food supply. The proportional risk was derived based on the number of BSE-reported cattle in a given year from 1980 through 1996 divided by the total number of BSE cases to date. The model generates categories (or bins) for Source Plasma donors by year of travel and estimates the risk more accurately by incorporating the information about proportional risk due to the BSE epidemic in the UK

In 1996 after the emergence of human vCJD cases the UK government implemented stringent food chain controls that decreased the number of high risk animals and high risk tissues containing BSE agent from entering the human food supply. In the early 1980s human exposure may have begun at a low level as BSE spread among the UK cattle population. The BSE epidemic expanded throughout the 1980s and peaked in 1992, then risk started to decrease as animal feed measures were implemented and more stringent human food chain controls were implemented in 1996. Today, a few hundred cases of BSE are identified in the UK annually – but it is unlikely that the animals or products from BSE-infected animals in the UK enter the human food supply. The model incorporates the changing dynamics of the BSE epidemic since 1980 and accounts for relative changes in the levels of human, specifically US plasma donors, exposure to the BSE agent and possible vCJD infection.

**Variable:** $y$ - Calendar year of travel.

**Variable:** $V_y$ - Number of visits by year to the UK by US travelers (in thousands)

**Data used in the model:** Number of visits by year to the UK by US travelers (UK Government Statistics, 2005).

The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

<u>Assumption used in the model</u>: US <u>Source Plasma</u> donors have similar travel patterns to the US blood donor population, which is assumed to be similar for the larger US population.

**Assumption used in the model:** It was assumed that no US traveler visited the UK more than once per year. This may potentially overestimate the vCJD risk for US plasma donors (because repeat travel by the

same donor is not addressed) and underestimate it in certain other cases (travelers who visit multiple times per year). FDA found no data that quantified the numbers of multiple visits or repeat visits by the same traveler that likely occurred for US donors with a history of UK travel.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 is represented by the equation:

$$V_{y/1996} = V_y / V_{1996} \qquad \text{(IV.C.1.a-9)}$$

**Variable:** ***age*** - Age of US plasma donors in groups of 5-year increments (e.g., 20-24 yrs, etc.) and 18-19 yr old age cohort..

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B. 1.) - the annual Source Plasma donations by age groups.

**Variable:** $DR_{S\text{-}UK(age)}$ - Number of Source Plasma donors who traveled to the UK from 1980 through 1996 by age group in five-year increments and 18-19 yr old age cohort.

**Assumptions used in the model:**

- The same percentage of Source Plasma donors traveled to UK as blood donors
- Frequencies of travels are similar among the donors of different age groups.
- Travel rates for the general US population are the same as the travel rate for blood and plasma donors.

The number of Source Plasma donors who traveled to the UK from 1980 to 1996 by age group is represented by:

$$DR_{S\text{-}UK(age)} = DR_{S(age)} \times Perc_{DR\text{-}UK} \qquad \text{(IV.C.1.a-5)}$$

**Variable:** $DR_{S\text{-}UK(age),y}$ - the number of Source Plasma donors who traveled to the UK in year $y$ by age group

Source Plasma donors with a history of travel to the UK among each age group ($DR_{S\text{-}UK(age)}$) was allocated to individual travel years based on the yearly distribution of visits to the UK by US travelers (UK National Statistics, 2005). The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

<u>Assumption used in the model</u>: US Source Plasma donors have similar travel patterns as the general US population and US blood donors.

The number of US Source Plasma donors who have traveled to the UK in year y between 1980-1996 is represented by the equation:

$$DR_{S-UK(age)y} = DR_{S-UK(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \quad \text{(IV.C.1.a-10)}$$

## A-IV. C. 1. a. ii. b. US Source Plasma donors with history of travel to the UK: Duration of travel by age group

There were no data that we are aware of that details the travel histories of Source Plasma donors in the US. Travel data for US blood donors was used to estimate travel patterns for Source Plasma donors after an adjustment for the frequency of travel based on the age of Source Plasma donors and the age-specific odds ratios for travel, which was obtained from 1980-1996 Blood Donor Travel Survey (TSEAC, 2000). The model used the data on the number of Source Plasma donors who have traveled to the UK in a specific year and subdivided those individuals into additional categories based on estimated duration of stay. The categories of duration of stay was estimated based on the Blood Donor Travel Survey (TSEAC, 2000). Use of blood donor travel data in the model implicitly assumes that travel rates are similar for both blood and plasma donors. However, it is thought that plasma donors likely travel less and are therefore at lower risk for vCJD, therefore the model results may overestimate the potential vCJD risk for plasma donors.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to UK from 1980-1996 based on the time they spent in the UK (same variable used above in section A-IV. C. 1. a. i.).

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1. i.).

**Variable:** $DR_{S-UK(age),y}$ - the number of Source Plasma donors who traveled to the UK in year $y$ by age group (calculated in A-IV. C. 1. a. ii. (a))

**Variable:** $Perc_{BIDR-UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to the UK (calculated in A-IV. C. 1. a. i. a)

**Variable:** $DR_{S-UK(age)y,i}$ - Number of Source Plasma donors within a specific age group that traveled to the UK in year $y$ for a duration of $i$ and is represented by the equation:

$$DR_{S-UK(age)y,i} = DR_{S-UK(age)y} \times Perc_{DR-UKi/UK} \quad \text{(IV.C.1.a-11)}$$

## A-IV. C. 1. a. ii. c. Number of US recovered plasma donors with a history of travel to the UK in a specific year from 1980 – 1996 by age group

Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year (or once every 54 days)– and on average we assumed a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable: $y$ – year of travel** (same variable used above in section A-IV. C. 1. a. ii. a) to the UK during the period 1980 - 1996 by US plasma donors.

**Variable: age – age groups of the population in five-year increments** (same variable used above in section A-IV. B. 1.).

**Variable:** $DR_{R(age)}$ - number of potential recovered plasma donors per year by age group (described in section A-IV. B. 2).

**Variable:** $Perc_{BIDR\text{-}UK(age)}$ (calculated in section A-IV.C.1.a.i.b.) - the percentage blood donors in each specific age group who have traveled to the UK between 1980-1996.

**Variable:** $DR_{R\text{-}UK(age)}$ – The number of recovered plasma donors who have traveled to the UK from 1980 through 1996 by age group and is represented by the equation:

$$DR_{R-UK(age)} = DR_{R(age)} \times Perc_{BIDR-UK(age)} \qquad \text{(IV.C.1.a-12)}$$

The model categorizes US recovered plasma donors by year of travel in order to estimate the potential vCJD risk more accurately by incorporating the information about BSE epidemic in the UK. The number of beef cattle affected each year by the BSE epidemic in the UK changed throughout the epidemic. Donors who visited the UK at the height of the BSE epidemic in 1992, we assumed, were more likely to be exposed to BSE agent (and vCJD risk) than a donor who visited in 1996 after food chain controls were implemented. The model accounts for this changing exposure potential by analyzing US donors who may have traveled to the UK for each year from 1980 through 1996. The number of recovered plasma donors who traveled to the UK was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers determined from UK travel data.

**Variable:** $DR_{R\text{-}UK(age)}$ - Number of recovered plasma donors traveled to the UK from 1980 through 1996 by age groups (calculated above).

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a. ii. a.).

**Data used in the model:** Number of visits by year to the UK by US travelers was determined using UK Government Statistics (2005).

**Assumption about variable:** US recovered plasma donors have a similar travel pattern as the US total population. The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during

the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

The number of recovered plasma donors who traveled to the UK in year *y* by age groups during the period from 1980-1996 is represented by the equation:

$$DR_{R-UK(age)y} = DR_{R-UK(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.a-13)}$$

## A-IV. C. 1. a. ii. d. US recovered plasma donors with history of travel to the UK: Duration of travel by age group

Recovered plasma donors who traveled to the UK in a specific year ($DR_{R-UK(age)y}$) in the years 1980-1996 were further partitioned in the model into subgroups or "bins" based on travel duration and by 5-year age groups and 18-19 yr old age cohort. Data on the percentage of blood donors who traveled to the UK since 1980 for a certain duration(s) (TSEAC 2000) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to the UK from 1980-1996 based on the time they spent in the UK (same variable used above in section A-IV. C. 1.)

**Variable:** $D_I$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R-UK(age)y}$ - The number of recovered plasma donors who traveled to the UK in year *y* by age group (calculated in A-IV. C. 1. a. ii. c)

**Variable:** $Perc_{BIDR-UKi/UK}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to the UK (calculated in A-IV. C. 1. a. i. a)

**Variable:** $DR_{R-UK(age)y,i}$ - The number of recovered plasma donors among an age group who have traveled to the UK in year, *y* ,for duration of $i$ and represented by the expression:

$$DR_{R-UK(age)y,i} = DR_{R-UK(age)y} \times Perc_{DR-UKi/UK} \qquad \text{(IV.C.1.a-14)}$$

## A-IV. C. 1. a. iii. US plasma donors with a history of travel to the UK: Adjustment of relative risk to account for variations in BSE risk by specific year and travel duration

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because the BSE epidemic in UK cattle and exposure of the human population to the BSE agent in the UK was greater than any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence, which is difficult to estimate. The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (TSEAC 2004). BSE was first diagnosed in the United Kingdom in 1986 and the epidemic peaked in 1992, a year when the risk of exposure to the BSE agent would have likely been highest for residents and visitors to the UK. Human exposure risk to the BSE agent would likely have decreased dramatically in 1996 with the culling of animals over 30 months of age from the food production system and the institution of food chain controls to prevent high risk tissues th might contain BSE agent from entering the food and animal food supplies. Presumably there were dramatic variations in the BSE exposure risk, and hence, the human vCJD infection risk that occurred from year to year. Therefore, the model adjusted the vCJD risk for US plasma donors with a history of extended travel or residence in the UK by multiplying by the proportional BSE risk per year (e.g., the BSE exposure risk in a given year compared to the total BSE risk since 1980). Additionally, the model included calculations on the estimated duration of UK travel or residence by US plasma donors based on US donor survey data (TSEAC 2000) to generate a more accurate vCJD risk estimate.

### A-IV. C. 1. a. iii. a. Variant CJD risk for individual UK residents from 1980 through 1996

**Variable:** $R_{UK}$- The accumulated vCJD risk per UK resident from 1980 through 1996.

**Assumption used in the model:** The UK population has the highest risk of exposure to BSE or vCJD, we assumed the average accumulated risk per UK individual is 1. Also, the relative risk for UK residents is 1, which is equivalent to the UK vCJD prevalence.

### A-IV. C. 1. a. iii. b. US plasma donors with a history of travel to the UK: Adjustment for the proportional individual BSE exposure risk for the UK population per year between 1980 to 1996.

The model calculates the risk and potential magnitude of BSE exposure for donors, in any given year in the UK since 1980, as a function of the number of BSE cases in a specific year divided by the total of all BSE cases since 1980.

**Variable:** $y$ – year of travel (same as variable used above in section A-IV. C. 1. a. ii. A.) by US plasma donor to the UK from 1980 to 1996.

**Variable:** $BSE_{UKy}$- The annual number of reported BSE cases in the UK since 1986 (OIE, 2005).

**Variable:** $R_{UKy}$- Proportional BSE exposure risk in the UK by specific year from 1980 to 1996.

**Assumptions used in the model:**

- The BSE exposure risk, and hence, most of the vCJD risk in the UK occurred largely between 1980 and 1996.
- The vCJD risk in the UK was assumed to be negligible after 1996, when stringent food chain controls were put in place to prevent contamination of beef with high risk tissue.
- The yearly rate of the human exposure risk to the BSE agent in the UK is proportional to the number of reported BSE annual cases in the UK
- The vCJD risk is additive for each year of residency during the specific time period.
- A person residing for five or more years during the time period between 1980 and 1996 in the UK is assumed to have a relative risk of 1 (or 100%), i.e., a probability of vCJD infection that is the same as that of the entire UK population.

The proportional BSE risk in the UK per specific year prior to 1997 is represented by the equation:

$$R_{UKy} = R_{UK} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.a-15)}$$

## A-IV. C. 1. a. iii. c. US plasma donors with a history of travel to the UK: BSE exposure risk and vCJD risk in year *y* for a period of *i*, during the period from 1980 to 1996.

**Variable:** $R_{DR\text{-}UKy,I}$- The potential vCJD risk of an individual US donor who traveled to the UK in specific year during the period 1980-1996 for a specific duration.

The potential vCJD risk for the US plasma donor subpopulation that traveled to the UK in a specific year for a specific duration was calculated using a pro-rated monthly rate, which was calculated based on the proportional BSE exposure risk in the UK in the specific year. The blood donor travel survey conducted by the American Red Cross (TSEAC 2000) collected data on the accumulated stay of donors in the UK from 1980 through 1996, which, for simplicity, was assumed to be the duration of a single, consecutive stay, when calculating the risk.

**Assumptions used in the model:**

- Risk of vCJD infection is proportional to the duration of the stay in the UK during the period 1980-1996
- All travelers evaluated completed a single, consecutive stay

As mentioned earlier, any US plasma donor with 5 years or more of accumulated stay in the UK is assumed to have average risk of 1, a risk equal to the average risk of an UK resident and equal to the UK vCJD prevalence.

The BSE exposure risk for US plasma donors with a stay less than or equal to one year – is represented by the equation:

$$R_{DR\text{-}UKy,I} = (R_{UKy}/12) \times D_i \qquad \text{(IV.C.1.a-16)}$$

for $i_{upper} \leq 1$ years;

The BSE exposure risk for US plasma donors with a stay less than five years but greater than or equal to one year is represented by the equation:

$$R_{DR-UKy,i} = (Average(R_{UKy} : R_{UK(y+Roundup(i_{upper},))}/12) \times D_i \quad \text{(IV.C.1.a-17)}$$

for 5 years $< i_{upper} \geq 1$ year;

The BSE exposure risk for US plasma donors with a stay greater than or equal to five – is represented by the equation:

$$R_{DR-UKy,i} = 1 \quad \text{(IV.C.1.a-18)}$$

for $i_{upper} \geq 5$ years

## A-IV. C. 1. a. iv. US plasma donors with a history of travel to the UK: Probability of potential infection with vCJD based on duration of travel to the UK and age

This section describes the portion of the model that estimates the probability that a US plasma donor in a specific age group, who traveled to the UK for a specific duration during the time-span of 1980 through 1996, was infected with vCJD.

**Variable:** $Pr_{vCJD-UK(age)}$ – the probability of vCJD infection per individual UK resident of a specific age group

**Variable:** $Prev_{Asym-vCJD(age)}$- Prevalence of asymptomatic vCJD infection in the UK for each age groups in five-year increments (e.g., 20-24 yrs, etc.) and the 18-19yr old group (calculated in A-IV.A.3.b.).

$$Pr_{vCJD-UK(age)} = Prev_{Asym-vCJD(age)}/100000 \quad \text{(IV.C.1.a-19)}$$

**Variable:** $Pr_{vCJD-DR-UK(age)y,i}$ – The probability of infection for individual US plasma donor of a specific age group who had traveled to the UK in a specific year for a specific duration

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$Pr_{vCJD-DR-UK(age)y,i} = Pr_{vCJD-UK(age)} \times R_{DR-UKy,i} \quad \text{(IV.C.1.a-20)}$$

## A-IV. C. 1. a. v. Number of all US pdFVIII plasma donors with history of travel to the UK and potentially infected with vCJD

This section of the model estimates the total number of all US plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996. To derive the total number of donors the model separately estimates the number of potentially infected Source Plasma donors and potentially infected recovered plasma donors (described in the subsequent sections below) and sums the two.

APPENDIX A

**A-IV. C. 1. a. v. a. Number US Source Plasma donors with history of travel to the UK and potentially infected with vCJD during travel to the UK**

Plasma is collected from Source Plasma donors in a process called plasmapheresis in which an average of approximately 700 milliliters of plasma are collected. Source Plasma donors donate an average of 14 times per year, but can donate up to 48 times per year.

This component of the model estimates the number of US Source Plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}UK(age)y,i}$ - Number of Source Plasma donors potentially infected with vCJD during travel to the UK during 1980-1996 by age, year and duration of travel.

$$DR_{vCJD-S-UK(age)y,i} = Bionomial(DR_{S-UK(age)y,i}, \Pr_{vCJD-DR-UK(age)y,i}) \quad \text{(IV.C.1.a-21)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UKy}$ - Number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK.

$$DR_{vCJD-S-UK} = \sum_{Age=18-19yrs}^{50-54yrs} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-S-UK(age)y,i} \quad \text{(IV.C.1.a-22)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Defy}$ - Number of Source Plasma donors potentially infected with vCJD in year y and not deferred by current policy.

$$DR_{vCJD-S-UK-defy} = \sum_{Age=18-19yrs}^{50-45yrs} \sum_{i=3-5months}^{>=5years} DR_{vCJD-S-UK(age)y,i} \quad \text{(IV.C.1.a-23)}$$

Current deferral policy (FDA 2002) defers individuals who have history of travel to the UK from 1980 through 1996 for an accumulated residence of 3 months or more from donating blood and plasma. The number of potentially infected donors who meet deferral criteria was calculated by equation:

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Res}$ - Residual risk due to the number of Source Plasma donors potentially infected with vCJD not deferred by current policy

$$DR_{vCJD-S-UK-Resy} = \sum_{Age=18-19}^{50-54yrs} DR_{vCJD-S-UK(age)y,i=1day-3months} \quad \text{(IV.C.1.a-24)}$$

## A-IV. C. 1. a. v. b. Number of US Source Plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

Perhaps the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD-infected donor that contained infectious vCJD agent in their blood at the time of

donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of Source Plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $y$ - The calendar year in which a plasma donor traveled and infected with vCJD.

**Assumption used in the model:** This risk assessment assesses the risk for pdFVIII product made in 2002.

**Variable:** $T_{Inf\text{-}2002y}$ - Time Period between infection/travel and year of 2002 when the plasma was collected

**Variable:** $Pr\text{-}LH_y$ - Probability the disease is in the last half incubation period of the disease (and donor is prionemic), if infected in year y

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected.

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.a-25)}$$

For an individual to have vCJD agent present in their blood and plasma (prionemic) in 2002, the elapsed period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or greater than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent the time between events, in this case the time from infection to the occurrence of symptomatic disease. The distribution is defined by two parameters, one that produces the shape of the curve and a second that generates the scale between events, which in this case is the mean incubation period of 14 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual has vCJD agent present in their blood and plasma in year 2002 (the baseline year of the model) was calculated by the expression:

Cumulative frequency of Gamma (4.7, 3.6), at x = 2×(1997-y)

**Variable:** $DR_{vCJD\text{-}S\text{-}UKy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK.

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and were in the last half incubation period of the disease in 2002 at the time of donation.

$$DR_{vCJD-S-UK-LHy} = Binomial(DR_{vCJD-S-UKy}, Pr_{LH-y}) \qquad \text{(IV.C.1.a-26)}$$

APPENDIX A

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}defy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and met deferral criteria

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}def\text{-}LHy}$ - Total number of Source Plasma donors in the last half incubation period of the disease (prionemic) and met current deferral criteria (FDA 2002).

$$DR_{vCJD\text{-}S\text{-}UK\text{-}Def\text{-}LHy} = Binomial(DR_{vCJD\text{-}S\text{-}UK\text{-}Defy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-27)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Resy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in the UK and did not meet deferral criteria

**Variable:** $DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}LHy}$ - Total number of Source Plasma donors in the last half of the incubation period of the disease who did not meet current deferral criteria (FDA 2002).

$$DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}LHy} = Binomial(DR_{vCJD\text{-}S\text{-}UK\text{-}Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-28)}$$

## A-IV. C. 1. a. v. c. Number of US recovered plasma donors with history of travel to the UK and potentially infected with vCJD

Recovered plasma donors donate whole blood from which the plasma is separated out (or recovered). Like blood donors recovered plasma donors donate an average of 1.7 times per year but can donate up to 6 times per year. The model assumes the average amount of plasma in a recovered plasma unit is approximately 200 milliliters.

This component of the model estimates the number of US recovered plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}R\text{-}UK(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to the UK during 1980-1996 by age, year and duration of travel

$$DR_{vCJD\text{-}R\text{-}UK(age)y,i} = Bionomial(DR_{R\text{-}UK(age)y,i}, \Pr_{vCJD\text{-}DR\text{-}UK(age)y,i}) \quad \text{(IV.C.1.a-29)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\,y}$ - Total number of recovered plasma donors potentially infected with vCJD in year y

$$DR_{vCJD\text{-}R\text{-}UKy} = \sum_{Age=18-19}^{50-54} \sum_{i=1day-3months}^{>=5years} DR_{vCJD\text{-}R\text{-}UK(age)y,i} \quad \text{(IV.C.1.a-30)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Defy}$ - Number of recovered plasma donors potentially infected with vCJD in year y and deferred by current policy

$$DR_{vCJD\text{-}R\text{-}UK\text{-}Defy} = \sum_{Age=18-19}^{50-54} \sum_{i=3-5months}^{>=5years} DR_{vCJD\text{-}R\text{-}UK(age)y,i} \quad \text{(IV.C.1.a-31)}$$

**Variable:** $DR_{vCJDS\text{-}UK\text{-}Resy}$ - Residual risk due to the number of recovered plasma donors potentially infected with vCJD not deferred by current policy and represented by the equation:

$$DR_{vCJD-R-UK-resy} = \sum_{Age=18-19}^{50-54} DR_{vCJD-R-UK(age)y,l=1day-3months} \qquad \text{(IV.C.1.a-32)}$$

## A-IV. C. 1. a. v. d. Number of US recovered plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

As discussed in the sections above the most critical determinant in the model of whether exposure occurs is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (e.g., was prionemic) at the time of donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially have infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual will have vCJD agent in blood and plasma (prionemic) in year 2002

**Variable:** $DR_{vCJD\text{-}R\text{-}UKy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK (calculated in A-IV. C. 1. a. v. b)

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and in the last half incubation period of the disease.

$$DR_{vCJD-R-UK-LHy} = Binomial(DR_{vCJD-R-UKy}, \Pr_{LH-y}) \qquad \text{(IV.C.1.a-33)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and met deferral criteria (calculated in A-IV. C. 1. a. v. c)

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}def\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who met deferral criteria.

$$DR_{vCJD-R-UK-Def-LHy} = Binomial(DR_{vCJD-R-UK-Defy}, \Pr_{LH-y}) \qquad \text{(IV.C.1.a-34)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Resy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in the UK and did not meet deferral criteria (calculated in A-IV. C. 1. a. v. c).

**Variable:** $DR_{vCJD\text{-}R\text{-}UK\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who did not meet deferral criteria

$$DR_{vCJD-R-UK-Res-LHy} = Binomial(DR_{vCJD-R-UK-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.a-35)}$$

## A-IV. C. 1. a. v. e. Number of all US plasma donors with a history of travel to the UK and potentially infected with vCJD

This section sums the total number of all US plasma donors, predicted by the model to donate to plasma pools used in manufacturing pdFVIII made from plasma collected in the US. This includes Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD and arrives at an estimate of the total number of US donors potentially infected with vCJD.

**Variable:** $DR_{vCJD\text{-}UK}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK

$$DR_{vCJD-UK} = \sum_{y=1980}^{1996} DR_{vCJD-S-UKy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UKy} \quad \text{(IV.C.1.a-36)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Def}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and met deferral criteria

$$DR_{vCJD-UK-Def} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Defy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Defy} \quad \text{(IV.C.1.a-37)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Res}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and did not meet deferral criteria

$$DR_{vCJD-UK-Res} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Resy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Resy} \quad \text{(IV.C.1.a-38)}$$

## A-IV. C. 1. a. v. f. Total of all US plasma donors with a history of travel to the UK and potentially infected and with vCJD agent present in their blood

Again, whether a donor contains vCJD agent in their blood is a pivotal calculation for the model since a donation from such an individual would contain vCJD agent that may find its way into a large plasma pool of thousands of donations that are used to manufacture pdFVIII. This section sums the number of US Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD

and contain vCJD agent in their blood and arrives at an estimate of the total number of US donors potentially infected with vCJD and prionemic.

**Variable:** $DR_{vCJD\text{-}UK\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease

$$DR_{vCJD-UK-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-LHy} \quad \text{(IV.C.1.a-39)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Def\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease and met deferral criteria

$$DR_{vCJD-UK-Def-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Def-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Def-LHy} \quad \text{(IV.C.1.a-40)}$$

**Variable:** $DR_{vCJD\text{-}UK\text{-}Res\text{-}LH}$- Total number of plasma donors in the last half incubation period of the disease and did not meet deferral criteria

$$DR_{vCJD-UK-Res-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-UK-Res-LHy} + \sum_{y=1980}^{1996} DR_{vCJD-R-UK-Res-LHy}$$

## A-IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

### A-IV.C.1.b.i. US plasma donors with a history of travel to France: Percentage of donors and travel duration

In this portion, blood donors are characterized by frequency and duration of travel to France since 1980. The risk of vCJD infection is a function of exposure to the BSE agent and is assumed to be proportional to the amount of time spent, or duration of travel, in France since 1980. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 5 years) in France since 1980, and to derive the frequencies for various durations of travel for 5 years or more. Since the baseline year to estimate potential vCJD risk for US donors in our model was 2002 trends in the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) were extrapolated from the year 1997 to 2002 when necessary to estimate potential travel characteristics and risk beyond 1996. The period of 5 years or more corresponds to the length of time in the current policy that defers donors who traveled to or resided in France. The travel survey data on blood donors pose a limitation because the survey was conducted on whole blood donors and may not exactly reflect the travel histories of plasma donors. Unfortunately, to our knowledge there is not travel data available on plasma donors. Therefore, we assumed that plasma donor travel characteristics to the France since 1980 are similar to those of whole blood donors and used this information in the FDA risk assessment. Some may argue that plasma donors travel less frequently than their blood donor counterparts so use of data on blood donors may overestimate the risk.

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC 2000).

**Variable:** $i$ - The duration interval used to group donors who had traveled to France from 1980-1996 based on the quantity of time spent in France during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (months) for interval $i$ representing the duration of travel or residence by US donors in France during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}FRI}$ – The cumulative percentage of blood donors who traveled to France within duration interval $i$ or longer.

**Data used in the model:** Travel data for US blood donors was obtained from a blood donor survey conducted by the American Red Cross and presented at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC, 2000).

**Variable:** $Perc_{BIDR\text{-}FRI}$ - Percentage of blood donors who have traveled to France within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}FRI}$

**Variable:** $Perc_{BIDR\text{-}FRI/FR}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France is represented by the equation:

$$Perc_{BIDR-FRI\,/\,FR} = (Perc_{BIDR-FRI} / CumPerc_{BIDR-FR,t>1day-1month}) \times 100\% \qquad \text{(IV.C.1.b-1)}$$

## IV. C. 1. b. Estimation of the number of US plasma donors with a history of extended travel to France potentially infected and vCJD agent is present in the blood

## A-IV.C.1.b.ii. Number of US plasma donors with a history of travel to France by age group, year of travel and duration of travel

This part of risk assessment calculates the annual number of US source and recovered plasma donors that traveled to France by specific year(s) and for a specific duration of time since 1980 by age. For the purposes of our analyses we grouped all donors and donors who traveled to France since 1980 into age groups of five year increments (20 – 24yrs, 25 – 29 yrs, etc) and the two year cohort for 18 and 19 years of age.

We estimated the frequency of donor travel by age group based on travel data for the US population and donor survey data. First, the total number of blood donors who traveled to France between 1980 and 1996 was estimated by multiplying the total number of US blood donors in a specific year by the percentage of donors (from survey data) who traveled to France. The percentage of donors in each age group that traveled to France since 1980 was calculated based on the total annual number donors who traveled to France since 1980 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel. Finally, the information on the number of donors from each age group who traveled was

calculated by year and duration of travel. The risk of a US donor acquiring vCJD is a function of duration of the stay, as well, the year(s) (since 1980) they resided in France.

- **Calculation of the annual number of blood donors who traveled to France from 1980 through 1996**

**Variable :** $DR_{BI}$-The annual total number of potential blood donors in the US

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR-FR}$ - The total percentage of US blood donors who traveled to France during the period from 1980 through 1996.

**Data used in the model:** Approximately, 15.6% of US blood donors reported a history of travel to France during the period from 1980 through 1996, based on the travel data of US blood donors (TSEAC, 2000).

**Variable:** $DR_{BI-FR}$ -Total number of blood donors who have traveled to France during 1980 and 1996

$$DR_{BI-FR} = DR_{BI} \times Perc_{BIDR-FR} \qquad \text{(IV.C.1.b-2)}$$

Then, the percentage donors in each age group that traveled to France between 1980 and 1996 was calculated based on the total number donors who traveled to France, the number of donors from each age group and the odds ratio of travel for each age group.

**Variable:** $DR_{BI(age)}$- Annual number of blood donors from each age group (calculated A-IV.C.1.a)

**Variable:** $Perc_{BIDR-FR(age)}$ - The percentage of US blood donors of an age group who traveled to France during the period from 1980 through 1996.

Total number of blood donor who traveled to France equals to the sum of donors from all age groups who have ever traveled to France:

$$DR_{BI-FR} = \sum_{age=18-19}^{65-69} (Perc_{BIDR-FR(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.b-3)}$$

**Variable:** $Odd_{T(age)}$- Odds ratios of each age group for the likelihood of travel compared to the group of age 18-19 year-olds.

**Data used in the model:** The odds ratio of travel for each age group was derived from the travel data obtained from 1980-1996 blood donor travel survey (TSEAC 2000). An odds ratio of 1 was assigned to donor group of age 18-19. Odds ratio of other age group represents the frequency of travel of those age groups compared to the group of age 18-19

$$Perc_{BIDR-FR(age)} = Odd_{T(age)} \times Perc_{BIDR-FR(18-19)} \quad \text{(IV.C.1.b-4)}$$

Replacing equation IV.C.1.b-4 for $Perc_{BIDR-FR(age)}$ in equation IV.C.1.b-3, percentage blood donors from age group of 18-19 who have traveled to France can be calculated by following equation:

$$Perc_{BIDR-FR(18-19)} = DR_{BI-FR} / \sum_{age=18-19}^{65-69}(Odd_{T(age)} \times DR_{BI(age)}) \quad \text{(IV.C.1.b-5)}$$

Then, the percentage of blood donors from other age groups who have traveled to France can be calculated using equation IV.C.1.b-4 (above).

## A-IV. C. 1. b. ii. a. Number of US Source Plasma donors with a history of travel to France by age group and specific year of travel

This section of the model computes the number of US Source Plasma donors who traveled to France based on annual total of Source Plasma donors and the estimated percentage of Source Plasma donors who have traveled to France between 1980 and 1996. Travel after 1996 was extrapolated from the percentage donors who travel between 1980 and 1996 and yearly travel data. To factor in travel frequency associated with donor age, the number of donors who have traveled was calculated by age groups.

**Variable:** ***age*** - Age of US plasma donors in groups of 5-year increments (20 – 24 yrs, 25 – 29yrs, etc) and the 18-19 year old cohort.

**Variable:** $DR_{S(age)}$ (calculated in section A-IV.B.1.b.) - the annual Source Plasma donations by age groups.

**Variable:** $Perc_{BIDR-FR(age)}$ (calculated in section A-IV.C.1.b.i..b.) - the percentage blood donors from an age group who have traveled to France between 1980-1996

**Variable:** $DR_{S-FR(age)}$ - Number of Source Plasma donors who traveled to France from 1980 through 1996 by age group.

**Assumptions used in the model:** The percentage of Source Plasma donors who traveled to France is the same as blood donors of the same age group.

The number of Source Plasma donors who traveled to France from 1980 to 1996 by age group is represented by:

$$DR_{S-FR(age)} = DR_{S(age)} \times Perc_{BIDR-FR} \quad \text{(IV.C.1.b-6)}$$

The model assumes that the risk that a traveler was exposed to the BSE agent in France is associated with the magnitude of the BSE epidemic in the UK in the year of travel. Major exposure risk in France was presumably from consumption of beef imported from the UK that was produced from cattle infected with BSE. The model breaks down Source Plasma donors by year of travel, in order to estimate the potential

US donor vCJD risk more accurately by incorporating the information about the magnitude of the BSE epidemic in the UK in a given year.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year *y* compared to the number of visits in 1996 (calculated in A-IV. C. 1. a. ii. a.).

**Variable:** $DR_{S\text{-}FR(age)y}$ - Number of Source Plasma donors with history of travel to France in year *y* by age groups

The number of Source Plasma donors who have traveled to France was allocated to individual travel year based on the yearly distribution of visits to France.

$$DR_{S-FR(age)y} = DR_{S-FR(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.b-7)}$$

for travel during 1980-1996;

$$DR_{S-FR(age)y} = DR_{S-FR(age)1996} \times V_{y/1996} \qquad \text{(IV.C.1.b-8)}$$

for travel after 1996

**Assumption used in the model:** The yearly frequency and distribution of travel to France by US donors was ascertained from UK travel data and the blood donor survey data (TSEAC 2000). Therefore, in calculating the US donor risk for vCJD, donors the yearly distribution of travel visits by age groups was adjusted to account for the requirement that donors be 18 years of age or older. The model also accounted for the fact that some younger donors born during the period 1980 to 1986 may not have been born and would have essentially a zero chance of being exposed to BSE agent. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. b. ii. b. Number of US Source Plasma donors and duration of travel to France by age group

There are no data detailing the travel histories of Source Plasma donors available. Travel data for blood donors was used for Source Plasma donors after an adjustment for the frequency of travel based on the age of Source Plasma donors and the age-specific odds ratios for travel, which were obtained from 1980-1996 Blood Donor Travel Survey (TSEAC, 2000).

The model further subdivides the number of Source Plasma donors who traveled to France in a specific year by duration of stay. While there were no specific travel data detailing travel patterns to France since 1980 available for US Source Plasma donors, data on travel patterns for whole blood donors was used as a proxy. Donor travel in the model was subdivided into categories based on the percentage of blood donors

who traveled to France for certain durations (Watanabe, 2000) (>5yrs, ≤5yrs, etc) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to France from 1980-1996 based on the time they spent in France (same variable used above in section A-IV. C. 1. a. i.).

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1. i.).

**Variable:** $DR_{S\text{-}FR(age),y}$ - the number of Source Plasma donors who traveled to France in year $y$ by age group (calculated in A-IV. C. 1. b. ii. )

**Variable:** $Perc_{BIDR\text{-}FR/FR}$- The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France (calculated in A-IV. C. 1. b. i. )

**Variable:** $DR_{S\text{-}FR(age)y,i}$ - Number of Source Plasma donors among an age group who have traveled to France in year $y$ for a duration of $i$ and is represented by the equation:

$$DR_{S-FR(age)y,i} = DR_{S-FR(age)y} \times Perc_{DR-FRi/FR} \qquad \text{(IV.C.1.b-9)}$$

## A-IV. C. 1. b. ii. c. Number of US recovered plasma donors with history of travel to France in a specific year between 1980 and 1996 – and by age group

Recovered plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year – and on average a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable:** $y$ **– Year of travel** (same variable used above in section A-IV. C. 1. b. ii. a) to France since 1980 by US plasma donors.

**Variable:** age – Age of the population by five-year increments (same variable used above in section A-IV. B. 1.).

**Variable:** $DR_{R(age)}$ - Number of potential recovered plasma donors per year by age group (described in section A-IV. B. 2).

**Variable:** $Perc_{BIDR\text{-}FR(age)}$ (calculated in section A-IV.C.1.b.i.)- The percentage blood donors from an age group who have traveled to France between 1980-1996

**Variable:** $DR_{R\text{-}FR(age)}$ - Number of recovered plasma donors who traveled to France from 1980 through 1996 by age groups and is represented by the equation:

$$DR_{R-FR(age)} = DR_{R(age)} \times Perc_{BIDR-FR(age)} \quad \text{(IV.C.1.b-10)}$$

The risk a traveler was exposed to BSE in France is proportional to the magnitude of the BSE epidemic in the UK in the year of travel. Because the major exposure risk in France is assumed to be from consumption of BSE contaminated beef imported from the UK , the model subdivides recovered plasma donors and groups them by year of travel. This provides a more precise estimate of the risk by incorporating the specific information on donor and donation characteristics and details that better capture the dynamics of the BSE epidemic in the UK on a year by year basis.

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a)

**Variable:** $DR_{R\text{-}FR(age),y}$ - Number of recovered plasma donors with a history of travel to France in year $y$ by age groups

Number of recovered plasma donors who have traveled to France was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers.

$$DR_{R-FR(age)y} = DR_{R-FR(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \quad \text{(IV.C.1.b-11)}$$

for travel during 1980-1996;

$$DR_{R-FR(age)y} = DR_{R-FR(age)1996} \times V_{y/1996} \quad \text{(IV.C.1.b-12)}$$

for travel after 1996

**Assumptions used in the model:** The yearly distribution of travel to France by US donors is similar to the yearly distribution of travel to the UK by US donors- this is based on travel data and the blood donor survey (TSEAC 2000). The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. b. ii. d. US recovered plasma donors with a history of travel to France and specific year of travel and duration of travel by age group

Recovered plasma donors who traveled to France in a specific year ($DR_{R\text{-}FR(age)y}$) since 1980 were further partitioned into the subgroups based on travel duration and by 5-year age groups. Data on the percentage

of blood donors who traveled to France since 1980 for certain duration(s) (TSEAC, 2000) was used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to France from 1980-1996 based on the time they spent in France (same variable used above in section A-IV. C. 1.)

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R\text{-}FR(age)y}$ - The number of recovered plasma donors who traveled to France in year $y$ by age group (calculated in A-IV. C. 1. b. iv. c.)

**Variable:** $Perc_{BIDR\text{-}FR/FR}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to France (calculated in A-IV. C. 1. b. ii. a.)

**Variable:** $DR_{R\text{-}FR(age)y,i}$ - Number of recovered plasma donors among an age group who have traveled to France in year, $y$ ,for duration of $i$.

$$DR_{R-FR(age)y,i} = DR_{R-FR(age)y} \times Perc_{DR-FR/FR} \qquad \text{(IV.C.1.b-13)}$$

## A-IV.C. 1. b. iii. US plasma donors with history of travel to France: Adjustment of the relative risk for France based on the magnitude of the UK BSE risk and by travel duration in a specific year

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because exposure to BSE in the UK was greater than any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence which is difficult to estimate. The relative risk value for France is 0.05 (or 5% of the UK risk). The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (TSEAC 2004). France received meat and bone meal from the UK during the BSE epidemic and approximately 5% of its beef was imported from the UK as of August 2006, France reported 20 cases of vCJD in its human population. Additionally, the model included calculations on the estimated duration of travel in France or residence by US plasma donors to generate a more accurate vCJD risk estimate. Current US vCJD geographic deferral policy defers donors with a history of residence in France for a period of 5 years or more since 1980.

## A-IV. C. 1. b. iii. a. US plasma donors with a history of travel to France: Average accumulated risk of residence since 1980

**Variable:** $R_{FR}$ – The cumulative risk of individual residents of France from 1980 till present; assuming that the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The average cumulative risk of a resident of France since 1980 is 0.05 relative to 1, the average accumulated risk of UK individual since 1980, based on UK beef imports, vCJD cases and indigenous BSE in France

**A-IV. C. 1. b. iii. b. US plasma donors with history of travel to France: Proportional risk of individual resident per year since 1980**

**Variable:** $y$ – **year** (same variable used in A-IV. C. 1. a. iv.)

**Variable:** $BSE_{UKy}$ (same variable used in A-IV. C. 1. a. iii.)

**Variable:** $BSE_{FRy}$ - Annual numbers of reported BSE cases in France including indigenous and imported cases

**Data used in the model:** Data on the annual number of reported BSE cases in France was obtained from the World organization for animal health (OIE) (2005).

**Variable:** $R_{FRy}$ - Proportional risk in France in a specific year

**Assumptions used in the model:**

- Variant CJD Risk in France occurred starting in 1980 to the present. Evidence indicates that vCJD and BSE cases are still emerging.
- Risk is additive, and can be pro-rated in a yearly and further monthly basis.
- Yearly rate of the risk in France is proportional to the reported BSE annual cases (including indigenous and imported cases) in France.

## A-IV. C. 1. b. iii. c. US plasma donors with a history of travel to France: Potential vCJD risk for donors who traveled in year y for a period of i

**Variable:** $R_{DR\text{-}FR,i}$ - The risk of an individual US donor who traveled to France in a specific year with a specific duration of travel, assuming a cumulative risk for a UK individual from 1980 through 1996 is 1 (or equivalent to the UK vCJD prevalence for the entire UK population).

The vCJD risk for the US plasma donor subpopulation that traveled to France in a specific year for a specific duration was calculated by applying a prorated monthly rate of risk, which was calculated based on the yearly rate of the risk in France during the year of travel. The blood donor travel survey (TSEAC, 2000) collected information on the accumulated stay of the donors in France from 1980 through 1996, which, for simplicity, was used to calculate the duration of consecutive stay, when calculated the risk.

**Assumptions used in the model:**

- Risk is proportional to the duration of the stay
- All travelers have consecutive stays
- US plasma donor subpopulation having more than 5 years accumulated stay in France have average risk of 0.05, which is the same as the average risk of individual resident of France (equivalent to 5% of the UK vCJD prevalence).

$$R_{DR-FRy,i} = (R_{FRy}/12) \times D_i \qquad \text{(IV.C. 1. b-14)}$$

for $i < 1$ years;

$$R_{DR-FRy,i} = (Average(R_{FRy} : R_{FR(y+i)})/12) \times D_i \qquad \text{(IV.C.1.b-15)}$$

for 5 years $<i>=1$ year;

$$R_{DR-FRy,i} = 0.05 \qquad \text{(IV.C.1.b-16)}$$

for $i >= 5$ years

## A-IV.C. 1. b. iv. US plasma donors with history of travel to France: Probability of vCJD infection for donor of a specific age group, in a specific year, for a specific duration, i

This section describes the portion of the model that estimates the potential probability that a US plasma donor in a specific age group who traveled to France for a specific duration since 1980 was infected with vCJD.

**Variable:** $Pr_{vCJD-FR(age)}$ Probability of vCJD infection in an individual resident of France in a specific age.

**Variable:** $Pr_{vCJD-DR-FR(age)y,i}$ - Probability of infection for individual US plasma donor of a specific age group who have traveled to France in a specific year for a specific duration.

**Assumption about variable:** Probability of vCJD infection being proportional to the risk of exposure to the BSE agent and represented by the equation:

$$\Pr_{vCJD-DR-FR(age)y,i} = \Pr_{vCJD-UK(age)} \times R_{DR-FRy,i} \qquad \text{(IV.C.1.b-17)}$$

## A-IV.C. 1. b. v. Total number of all US plasma donors with a history of travel to France: Number potentially infected with vCJD

### A-IV.C. 1. b. v. a. Number of US Source Plasma donors with a history of travel to France and potentially infected with vCJD

Plasma is collected from Source Plasma donors in a process called plasmapheresis in which approximately 700 milliliters of plasma are collected. Source Plasma donors donate an average of 14 units per year, but can donate up to 48 times per year.

This component of the model estimates the number of US Source Plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}FR(age)y,i}$ - Number of Source Plasma donors potentially infected with vCJD during travel to France since 1980 by age, year and duration of travel.

$$DR_{vCJD-S-FR(age)y,i} = Bionomial(DR_{S-FR(age)y,i}, \Pr_{vCJD-DR-FR(age)y,i}) \quad \text{(IV.C.1.b-18)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FRy}$ - Number of Source Plasma donors potentially infected with vCJD in year y during travel/residence in France.

$$DR_{vCJD-S-FRy} = \sum_{Age=18-19\,yrs}^{50-54\,yrs} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-S-FR(age)y,i} \quad \text{(IV.C.1.a-19)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Defy}$ - Number of Source Plasma donors potentially infected with vCJD in year y and meet deferral criteria.

Current deferral policy defers individuals who have a history of travel to France since 1980 for an accumulated time of over 5 years from donating blood and plasma. The number of potentially infected donors who meet deferral criteria was calculated by equation:

$$DR_{vCJD-S-FR-Defy} = \sum_{Age=18-19}^{50-54\,yrs} DR_{vCJD-S-FR(age)y,i>=5\,years} \quad \text{(IV.C.1.a-20)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Resy}$ - Residual risk due to the number of Source Plasma donors potentially infected with vCJD in year y and not deferred by current policy

$$DR_{vCJD-S-FR-Resy} = \sum_{Age=18-19\,yrs}^{50-54\,yrs} \sum_{i=1-30days}^{>3-5\,years} DR_{vCJD-S-FR(age)y,i} \quad \text{(IV.C.1.a-21)}$$

## A-IV. C. 1. b. v. b. Number of US Source Plasma donors with history of travel to France and potentially infected and vCJD agent is present in the blood

Perhaps the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (i.e., was prionemic at the time of donation). Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of Source Plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** *y*-The calendar year in which a plasma donor traveled and infected with vCJD

**Assumption used in the model:** This risk assessment assesses the risk for pdFVIII product made in 2002 (but risk is assumed to be similar up to the year 2006).

**Variable:** $T_{Inf\text{-}2002y}$-Time Period between infection/travel and year of 2002 when the plasma was collected

**Variable:** $Pr\text{-}LH_y$-Probability the individual is in the last half incubation period of the disease, if infected in year y

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection and travel and 2002 when the plasma was collected

$$T_{Inf-2002\,y} = 2002 - y \qquad \text{(IV.C.1.b-22)}$$

For an individual to have vCJD agent present in their blood and plasma (prionemic) in 2002, the elapsed period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or less than the remaining half of incubation period of the disease; in another words, the incubation period of the disease should be equal to or less than twice as much as $I_{Inf\text{-}2002y}$.

**Assumption used in the model** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially, in this case infection, incubation period of the disease, etc. The distribution is defined by two parameters (or arguments) that produce the shape of the curve and generates a mean incubation period of 14 years and a median incubation period of 13 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual will be prionemic in the year 2002, was determined using the distribution:

Cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

Variable: $DR_{vCJD\text{-}S\text{-}FRy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France (calculated in A-IV. C. 1. b. v. a.)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and in the last half incubation period of the disease.

$$DR_{vCJD-S-FR-LHy} = Binomial(DR_{vCJD-S-FRy}, \Pr_{LH-y}) \quad \text{(IV.C.1.b-23)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}defy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and met deferral criteria (calculated in A-IV. C. 1. b. v. a)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}def\text{-}LHy}$ - Total number of Source Plasma donors in the last-half of the incubation period of the disease who met deferral criteria.

$$DR_{vCJD-S-FR-Def-LHy} = Binomial(DR_{vCJD-S-FR-Defy}, \Pr_{LH-y}) \quad \text{(IV.C.1.b-24)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Resy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during travel/residency in France and did not met deferral criteria (calculated in A-IV. C. 1. b. v. a)

**Variable:** $DR_{vCJD\text{-}S\text{-}FR\text{-}Res\text{-}LHy}$ - Total number of Source Plasma donors in the last half incubation period of the disease who did not met deferral criteria or were not deferred is represented by the equation:

$$DR_{vCJD-S-FR-Res-LHy} = Binomial(DR_{vCJD-S-FR-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.b-25)}$$

## A-IV.C. 1. b. v. c. Number of US recovered plasma donors with a history of travel to France and potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}R\text{-}FR(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to France since 1980 by age, year and duration of travel

$$DR_{vCJD-R-FR(age)y,i} = Bionomial(DR_{R-FR(age)y,i}, \Pr_{vCJD-DR-FR(age)y,i}) \quad \text{(IV.C.1.b-26)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}FRy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y:

$$DR_{vCJD-R-FRy} = \sum_{Age=18-191=1day-3months}^{50-54} \sum^{>=5years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.b-27)}$$

Current deferral policy defers individuals who have history of travel to France since 1980 for an accumulated residence of 5 years or more from donating blood and plasma. The number of potentially infected donors who meet the deferral criteria was calculated by equation:

$$DR_{vCJD-R-FR-Defy} = \sum_{Age=18-19}^{50-54\,yrs} DR_{vCJD-R-FR(age)y,t>=5\,years} \quad \text{(IV.C.1.b-28)}$$

**Variable:** $DR_{vCJD-S-FR-Resy}$- The residual risk due to the number of recovered plasma donors potentially infected with vCJD in year y and not deferred by current policy

$$DR_{vCJD-R-FR-Resy} = \sum_{Age=18-19\,yrs}^{50-54\,yrs} \sum_{i=1-30\,days}^{>3-5\,years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.b-29)}$$

## A-IV. C. 1. b. v. d. Number of US recovered plasma donors with history of travel to France and potentially infected and vCJD agent is present in the blood

As discussed in the sections on Source Plasma (above) the most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor who had infectious vCJD agent in their blood (i.e., was prionemic at the time of donation). Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH-y}$ -The probability an individual will have vCJD agent present in their blood or present (prionemic) at the time of donation in the year 2002 (calculated in A-IV.C.1.a.v. b.)

Variable: $DR_{vCJD-R-FRy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in France (calculated in A-IV. C. 1. b. v. c.)

**Variable:** $DR_{vCJD-R-FR-LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in France and in the last half incubation period of the disease.

$$DR_{vCJD-R-FR-LHy} = Binomial(DR_{vCJD-R-FRy,} \Pr_{LH-y}) \quad \text{(IV.C.1.b-30)}$$

**Variable:** $DR_{vCJD-R-FR-defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in France and met deferral criteria (calculated in A-IV. C. 1. b. v. c)

**Variable:** $DR_{vCJD-R-FR-def-LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who met deferral criteria and presumably were deferred from donation.

$$DR_{vCJD-R-FR-Def-LHy} = Binomial(DR_{vCJD-R-FR-Defy,} \Pr_{LH-y}) \quad \text{(IV.C.1.b-31)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}FR\text{-}Resy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in France and did not met deferral criteria and were likely not deferred from donation (calculated in A-IV. C. 1. b. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}FR\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease who did not meet deferral criteria and were likely not deferred from donation.

$$DR_{vCJD-R-FR-Res-LHy} = Binomial(DR_{vCJD-R-FR-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.b-32)}$$

## A-IV. C. 1. b. v. e. Number of all US plasma donors with history of travel to France and potentially infected with vCJD

This section sums the number of all US plasma donors, predicted by the model to donate to plasma pools used in manufacturing pdFVIII made from plasma collected in the US. This includes recovered plasma donors and Source Plasma donors, and generates an estimate for the total number of donors potentially infected with vCJD during extended travel to France since 1980.

**Variable:** $DR_{vCJD\text{-}FR}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in France

$$DR_{vCJD-FR} = \sum_{y=1980}^{1996} DR_{vCJD-S-FRy} + \sum_{y=1980}^{1996} DR_{vCJD-R-FRy} \quad \text{(IV.C.1.b-33)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Def}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in France and meet deferral criteria

$$DR_{vCJD-FR-Def} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Defy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Defy} \quad \text{(IV.C.1.b-34)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Res}$ - Total number of plasma donors potentially infected with vCJD during travel/residence in the UK and did not meet deferral criteria

$$DR_{vCJD-FR-Res} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Resy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Resy} \quad \text{(IV.C.1.b-35)}$$

## A-IV. C. 1. b. v. f. Total number of US plasma donors with history of travel to France and are potentially infected and vCJD agent is present in the blood

Again, whether a donor contains vCJD agent in their blood is a pivotal calculation in the model since a donation from such an individual would contain vCJD agent that may find its way into a large plasma pool of thousands of donations that are used to manufacture pdFVIII. This section sums the number of

APPENDIX A

US Source Plasma donors and recovered plasma donors predicted by the model to be infected with vCJD and contain vCJD agent in their blood and arrives at an estimate of the total number of US donors potentially infected with vCJD and who are prionemic.

**Variable:** $DR_{vCJD\text{-}FR\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease

$$DR_{vCJD-FR-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-S-FR-LHy} \quad \text{(IV.C.1.b-36)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Def\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease and met deferral criteria

$$DR_{vCJD-FR-Def-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Def-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Def-LHy} \quad \text{(IV.C.1.b-37)}$$

**Variable:** $DR_{vCJD\text{-}FR\text{-}Res\text{-}LH}$ - Total number of plasma donors in the last half incubation period of the disease and did not met deferral criteria

$$DR_{vCJD-FR-Res-LH} = \sum_{y=1980}^{2002} DR_{vCJD-S-FR-Res-LHy} + \sum_{y=1980}^{2002} DR_{vCJD-R-FR-Res-LHy} \quad \text{(IV.C.1.b-38)}$$

## A-IV.C. 1.c. Number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) potentially infected and vCJD agent is present in the blood

## A-IV.C.1.c.i. US recovered plasma donors with a history of travel to countries in Europe: Percentage of US donors and travel duration

In this portion of the FDA risk assessment, US blood (recovered plasma) donors are characterized by frequency and duration of travel to countries in Europe (other than the UK) during the period 1980-1996. The risk of vCJD infection is a function of exposure to the BSE agent and is assumed to be proportional to the amount of time spent, or duration of travel, in countries in Europe (other than the UK) since 1980. The FDA model used data from the National Blood Donor Travel Survey 1980-1996 (TSEAC 2000) to derive estimates of the percentages of US donors with a history of extended travel or residence ($\geq$ 5 years) in other countries in Europe (other than the UK and France) since 1980, and to derive the frequencies for various durations of travel for 5 years or more. The period of 5 years or more corresponds to the length of time in the current policy that defers blood (recovered plasma) donors who traveled to or resided in European countries (other than the UK).

**Data used in the model:** National Blood Donor Travel Survey 1980-1996 was conducted by the American Red Cross and presented at the TSEAC in 2000.

**Variable:** $i$ - The duration interval used to group donors who had traveled to countries in Europe from 1980-1996 based on the quantity of time spent in Europe (other than the UK and France) during the period from 1980 – 1996.

**Variable:** $D_i$ - The average duration of time (in months) for interval $i$ representing the duration of travel or residence by US donors in countries in Europe (other than the UK and France) during the period from 1980 – 1996.

**Variable:** $CumPerc_{BIDR\text{-}EUi}$ – The cumulative percentage of blood donors who traveled to countries in Europe (other than the UK and France) within duration interval $i$ or longer.

**Data used in the model:** Travel data for US blood donors was obtained from a blood donor survey conducted by the American Red Cross and presented at the TSEAC in 2000.

**Variable:** $Perc_{BIDR\text{-}EUi}$ - Percentage of blood donors who traveled to countries in Europe (other than the UK and France) within duration interval $i$. This variable was converted from $CumPerc_{BIDR\text{-}EUi}$

**Variable:** $Perc_{BIDR\text{-}EUi/EU}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to countries in Europe (other than the UK and France) is represented by the equation:

$$Perc_{BIDR-EUi/EU} = (Perc_{BIDR-EUi} / CumPerc_{BIDR-EU,i>1day-1month}) \times 100\% \qquad \text{(IV.C.1.e-1)}$$

The following portion of the risk assessment estimated the frequency of travel for each recovered plasma donor by age group based on travel data of blood donors. First, estimates for blood donors who traveled to countries in Europe (other than the UK and France) between 1980 and 1996 was calculated to generate the total number of US blood donors and percentages of donors who traveled. For the purposes of our analyses we grouped all donors and donors who traveled to countries in Europe since 1980 into age groups of five-year increments (20 – 24yrs, 25 – 29 yrs, etc) and for the 18 – 19 year old cohort. The percentage of donors in each age group that traveled to countries in Europe since 1980 was calculated based on the total annual number donors who traveled to Europe since 1980 compared to (or divided by) the total number of donors, and the age specific odds ratio for travel.

- **<u>Calculation of the annual number of blood donors who traveled to countries in Europe (other than the UK and France) from 1980 through 1996</u>**

**Variable :** $DR_{BI}$ - The annual total number of potential blood donors in the US.

**Data used in the model:** There are approximately 8 million individuals who donate blood each year in the United States (Westat, 2002).

**Variable:** $Perc_{BIDR\text{-}EU}$ - The total percentage of US blood donors who traveled to countries in Europe (other than the UK and France) during the period from 1980 through 1996.

**Data used in the model:** Approximately, 15.6% of US blood donors have histories of travel to countries in Europe (other than the UK and France) during the period from 1980 through 1996, based on the travel data of US blood donors (TSEAC, 2000).

**Variable:** $DR_{BI\text{-}EU}$ -Total number of blood donors who have traveled to countries in Europe (other than the UK and France) during the period 1980 - 1996.

$$DR_{BI-EU} = DR_{BI} \times Perc_{BIDR-EU} \qquad \text{(IV.C.1.c-2)}$$

Then, the percentage of donors in each age group who traveled to countries in Europe (other than the UK and France) between 1980 and 1996 was calculated based on the total number donors who traveled to countries in Europe, the number of donors from each age group, and the odds ratio of travel for each age group.

**Variable:** $DR_{BI(age)}$- Annual number of blood donors from each age group (calculated IV.C.1.a)

**Variable:** $Perc_{BIDR\text{-}EU(age)}$ - Annual percentage of US blood donors of an age group who traveled to countries in Europe during the period from 1980 through 1996.

Total number of blood donor who traveled to countries in Europe (other than the UK and France) equals to the sum of donors from all age groups who have ever traveled to Europe:

$$DR_{BI-EU} = \sum_{age=18-19}^{65-69} (Perc_{BIDR-EU(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.c-3)}$$

**Variable:** $Odd_{T(age)}$- The odds ratio for each five-year age group (e.g. 20-24 yrs, etc.) of travelers was compared to the group of age 18-19 yr olds.

**Data used in the model:** The odds ratios of travel to countries in Europe (other than the UK and France) for each age group was derived from the travel data obtained from 1980-1996 blood donor travel survey. An odds ratio of 1 was assigned to the donor group of age 18-19 yr olds. The odds ratios for the other age groups were generated by comparing the frequency of travel for those age groups to the group of age 18-19 yr-olds.

$$Perc_{BIDR-EU(age)} = Odd_{T(age)} \times Perc_{BIDR-EU(18-19)} \qquad \text{(IV.C.1.c-4)}$$

The percentage of blood donors from the age group of 18-19 yr olds who have traveled to countries in Europe (other than the UK and France) can be calculated by the following equation:

$$Perc_{BIDR-EU(18-19)} = DR_{BI-EU} / \sum_{age=18-19}^{65-69} (Odd_{T(age)} \times DR_{BI(age)}) \qquad \text{(IV.C.1.c-5)}$$

Then, the percentage blood donors from other age groups who traveled to Europe (other than the UK and France) can be calculated using the equation (A-IV.C.1.c-4).

## A-IV. C. 1. c. ii. US recovered plasma donors who traveled to countries in Europe: Total number by year of travel, duration of travel and by age group

This part of the risk assessment calculates the annual number of US recovered plasma donors who traveled to countries in Europe (other than the UK and France) since 1980. The number of donors from an age group who traveled was calculated by year and duration of travel. The risk of a US donor acquiring vCJD is a function of duration of the stay, as well as the year(s) (since 1980) they resided in Europe.

## A-IV. C. 1. c. ii.a. Number of US recovered plasma donors who traveled to Europe in a specific year between 1980 and 1996 by age group

This portion of the model estimates the potential vCJD risk for US recovered plasma donors with a history of travel to countries in Europe (other than the UK and France). Recovered plasma is derived from single units of Whole Blood as a by-product in the preparation of blood components from whole blood collection and is intended for further manufacturing. As expected, recovered plasma donor donation characteristics mirror those of whole blood donors. A recovered plasma donor can donate plasma a maximum of six times per year – and on average a recovered donation is approximately 200 milliliters (versus an average of 700 milliliters for a Source Plasma donation).

**Variable:** $y$ – year of travel (same variable used above in section A-IV. C. 1. b. ii. a.) to a country in Europe (other than the UK or France) since 1980 by US plasma donors.

**Variable: age** – Age groups of the population by five-year increments (same variable used above in section A-IV. B. 1.) and the two year cohort for 18 and 19 year olds for US recovered plasma donors who traveled to countries in Europe (other than the UK and France).

**Variable:** $DR_{R(age)}$ - Number of potential US recovered plasma donors per year by age group (described in section A-IV. B. 2.).

**Variable:** $Perc_{BIDR\text{-}EU(age)}$ (calculated in section A-IV.C.1.b.ii.b.)- The percentage US blood donors by age group who have traveled to countries in Europe (other than the UK and France) between 1980-1996.

**Variable:** $DR_{R\text{-}EU(age)}$ - Number of recovered plasma donors who traveled to countries in Europe (other than the UK and France). from 1980 through 1996 by age group and is represented by the equation:

$$DR_{R-EU(age)} = DR_{R(age)} \times Perc_{BIDR-EU(age)} \qquad \text{(IV.C.1.c-10)}$$

The risk a traveler was exposed to BSE in Europe is proportional to the magnitude of the BSE epidemic in the UK in the year of travel. Because the major exposure risk in Europe was assumed to be from consumption of beef contaminated with BSE agent imported from the UK. The model groups recovered plasma donors by year of travel. This provides a more precise estimate of the risk by incorporating the (age and year) specific information and details that better capture the dynamics of the BSE epidemic in the UK on a year by year basis. Travel for the years not covered by the UK National Survey 1997 to 2002 relied on extrapolation of trends from 1996 and before to estimate travel characteristics up to the baseline year of 2002 in the model (see equation A-IV.C.1.c-12 (below)).

**Variable:** $V_{y/1996}$ - The number of visits to the UK by US travelers in year $y$ compared to the number of visits in 1996 (calculated in A-IV.C.1.a.)

**Variable:** $DR_{R\text{-}EU(age),y}$ - Number of recovered plasma donors who traveled to Europe in year $y$ by age groups.

The number of recovered plasma donors who have traveled to countries in Europe (other than the UK and France) was allocated to individual travel year based on the yearly distribution of visits to the UK by US travelers.

$$DR_{R-EU(age)y} = DR_{R-EU(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.C.1.c-11)}$$

for travel during 1980-1996;

$$DR_{R-EU(age)y} = DR_{R} \times V_{y/1996} \qquad \text{(IV.C.1.c-12)}$$

for travel after 1996.

**Assumption used in the model:** The yearly distribution of travel to countries in Europe (other than the UK and France) by US recovered plasma donors is similar to the yearly distribution of travel to the UK by US travelers. The yearly distribution of travel visits by each age group was adjusted to account for the minimum age of 18 when a donor can donate plasma or blood. Therefore, in calculating the US donor risk for vCJD the yearly distribution of travel visits by each age group was adjusted to account for this requirement. The model adjusted the potential vCJD exposure for younger donors who were born during the period from 1980 to 1986 and would have essentially a zero chance of being exposed to the BSE agent in the years prior to their birth. Therefore, donors 18 years of age in 2002 were assumed to have zero exposure to the BSE agent prior to 1985, those 19 years of age in 2002 were assumed to have zero exposure prior to 1984, those 20 years of age in 2002 were assumed to have zero exposure prior to 1983, those 21 years of age in 2002 were assumed to have zero exposure prior to 1982, those 22 years of age in 2002 were assumed to have zero exposure prior to 1981. The model assumed that there was zero exposure of all donors prior to 1980.

## A-IV. C. 1. c. ii. b. Number of US recovered plasma donors who traveled to countries in Europe by specific year of travel and duration of travel by age group

Recovered plasma donors who traveled to countries in Europe (other than the UK and France) in a specific year ($DR_{R\text{-}EU(age)y}$) since 1980 were further partitioned into the subgroups in the model based on travel duration and by 5-year age groups and also the two year cohort of donors 18 and 19 years of age. Data on the percentage of blood donors who traveled to countries in Europe (other than the UK and France) since 1980 for a certain duration(s) (TSEAC, 2000) were used in this risk assessment.

**Variable:** $i$ - The duration interval used to group blood donors who had traveled to countries in Europe (other than the UK and France) from 1980-1996 based on the time spent (same variable used above in section A-IV. C. 1.).

**Variable:** $D_i$ - The average duration of time for interval $i$ (months) (same variable used above in section A-IV. C. 1.)

**Variable:** $DR_{R\text{-}EU(age),y}$ - The number of recovered plasma donors who traveled to countries in Europe (other than the UK and France) in year $y$ by age group

**Variable:** $Perc_{BIDR\text{-}EU/FEU}$ - The percentage of blood donors who traveled for a specific duration interval $i$ among all donors who have ever traveled to countries in Europe (other than the UK and France) (calculated in A-IV. C. 1. c. i).

**Variable:** $DR_{R\text{-}EU(age)y,i}$ - Number of recovered plasma donors within a specific age group who have traveled to countries in Europe (other than the UK and France) in year, $y$ ,for duration of $i$.

$$DR_{R-EU(age)y,i} = DR_{R-EU(age)y} \times Perc_{DR-EU/EU} \qquad \text{(IV.C.1.c-13)}$$

## A-IV.C. 1. c. iii. US recovered plasma donors who traveled to countries in Europe: Adjustment of relative risk for the proportion of exposure to the BSE agent per year and by duration of travel and by age group

As indicated in previous sections the FDA model assumed that the relative vCJD risk for UK residents residing for any five-year period or longer from 1980 through 1996 is assumed to have a value of 1, because exposure to BSE in the UK was greater than that of any other country. The relative risk value of 1 equates to 100% of the UK asymptomatic and symptomatic vCJD prevalence, which is difficult to estimate. Again, based on information in FDA guidance (2002), the relative risk value for France was assumed to be 0.05 (or 5% of the UK risk). The relative risk value is assigned based on factors such as domestic UK beef consumption, and the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (FDA 2002). Countries in Europe (other than the UK and France) received meat and bone meal from the UK during the BSE epidemic and approximately 1.5% of their beef was imported from the UK. Additionally, the model included calculations on the estimated duration of travel or residence in Europe by US plasma donors to generate a more accurate vCJD risk estimate. Current US vCJD geographic deferral policy defers blood donors with a history of residence in countries in Europe (other than the UK and France) for a period of 5 years or more since 1980; this policy does not include Source Plasma donors.

## A-IV. C. 1. c. iii. a. Number of US recovered plasma donors with a history of travel to countries in Europe: Average accumulated vCJD risk for donors since 1980, assuming that the average accumulated risk for a UK individual since 1980 is 1

This section calculates the number of US plasma donors with a history of travel to countries in Europe (other than the UK and France) and their average accumulated vCJD risk since 1980, assuming that the average accumulated risk for a UK individual since 1980 is 1.

**Variable:** $R_{EU}$ - Ac– The cumulative vCJD risk of an individual resident of countries in Europe (other than the UK and France) from 1980 till now; assuming the accumulated risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The average accumulated vCJD risk for travel to countries in Europe (other than the UK and France) by an individual resident since 1980 was assumed to be 0.015 relative to 1, the average accumulated risk of UK individual since 1980, based on UK. It was assumed that approximately 1.5% or less of the beef imports, imported into many countries since 1980 was imported from the UK. The 0.015 relative risk value also considers the number of human vCJD cases present and the presence of indigenous BSE in European countries (other than the UK and France) since 1980.

**A-IV. C. 1. c. iii. b. US plasma donors with a history of travel to countries in Europe: Proportional risk per individual resident per year since 1980**

**Variable:** $Y_{epi}$ –*y* Years of BSE epidemic in countries in Europe (other than the UK and France).

**Variable:** $BSE_{UKy}$ Annual numbers of reported BSE cases in the UK.

**Variable:** $BSE_{EUy}$ - Annual numbers of reported BSE cases in European countries other than the UK and France.

**Data used in the model:** Data on the annual number of reported cases of BSE in cattle in European countries was obtained from the Orgainization Internationale de Epizootics (OIE) (2005).

**Variable:** $R_{EUy}$ - Proportional vCJD risk for European countries (other than the UK and France) in a specific year

**Assumptions used in the model:**

- The risk of vCJD and BSE in European countries (other than the UK and France) has been present since 1980 and continues to present day. As of August 2006, very few cases of BSE continue to be reported in European countries (other than the UK and France)..
- The vCJD risk is assumed to be additive, and can be prorated on a yearly or monthly basis.
- The yearly rate of the vCJD risk in European countries (other than the UK and France) is proportional to the reported BSE annual cases in Europe (including indigenous and imported cases)

## A-IV. C. 1. c. iii. b. US recovered plasma donors with a history of travel to countries in Europe: Potential vCJD risk for an individual in year y for a period of i since 1980

**Variable:** $R_{DR\text{-}EUy_{n,i}}$ - Risk for individual US donors who traveled to countries in Europe other than the UK and France in a specific year for a specific duration, assuming the accumulative risk of a UK person resident in the years 1980 through 1996 is 1.

The vCJD risk for the US plasma donors with less than 5 years accumulated stay in Europe was calculated based on travel to European countries other than the UK and France in a specific year for a specific duration of travel. Potential exposure risk was calculated using a prorated monthly rate, which was calculated based on the yearly rate of the risk (1 month = 1/12 x yearly risk) in Europe during the year of travel. US plasma donors with a total accumulated stay in countries in Europe (other than the UK or France) of 5 years or more is assumed to have average risk of 0.015, which is the same as the risk of an individual citizen or long-term resident of a country in Europe (other than the UK or France). Information on duration of accumulated stays was collected in the blood donor travel survey; however, for simplicity we assumed all travel was consecutive. The blood donor travel survey (TSEAC 2000) collected information on the accumulated stay of US donors who stayed in Europe (other than the UK or France) from 1980 through 1996. For simplicity, these data were used to estimate the duration of consecutive stay, which was used to calculate the potential vCJD risk for recovered plasma donors.

Assumptions used in the model:

- Risk is proportional to the duration of the stay
- All the travelers' stays were assumed to be single and consecutive stays.
- US plasma donor subpopulation having 5 or more years accumulated stay in countries in Europe (other than the UK or France) have an average risk of 0.015, which is the same as the average risk of individual European resident.

For < 1 year;

$$R_{DR-EUy,i} = (Average(R_{EUy} : R_{EU(y+1)})/12) \times D_i \qquad \text{(IV. C. 1. c-14)}$$

for 5 years <i>=1 year;

$$R_{DR-EUy,i} = 0.015 \qquad \text{(IV. C. 1. c-15)}$$

for i>= 5 years

## A-IV. C. 1. c. iv. US recovered plasma donors with a history of travel to countries in Europe: Probability of vCJD infection for a donor of a specific age group, who traveled in a specific year for a specific duration, i

This section describes the portion of the model that estimates the potential probability that a US plasma donor in a specific age group, who traveled to countries in Europe (other than the UK or France) for a specific duration since 1980 was infected with vCJD.

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ – the probability of infection for an individual UK resident of a specific age group (calculated in A-IV. C. 1. a. iv.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}EU(age)y,i}$ - Probability of infection for an individual US plasma donor of a specific age group who have traveled to countries in Europe other than the UK and France in a specific year with specific duration.

Assumption used in the model: The probability of vCJD infection is proportional to the risk of exposure

$$\Pr_{vCJD-DR-EU(age)y,i} = \Pr_{vCJD-UK(age)} \times R_{DR-EUy,i} \quad \text{(IV.C.1.c-16)}$$

## A-IV.C. 1. c. v. Number of US recovered plasma donors with a history of travel to countries in Europe: Number potentially infected with vCJD.

**Variable:** $DR_{vCJD\text{-}R\text{-}EU(age)y,i}$ - Number of recovered plasma donors potentially infected with vCJD during travel to countries in Europe (other than the UK and France) since 1980 by age, year and duration of travel

$$DR_{vCJD-R-EU(age)y,i} = Bionomial(DR_{R-EU(age)y,i}, \Pr_{vCJD-DR-EU(age)y,i}) \quad \text{(IV.C.1.c-26)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EUy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y

$$DR_{vCJD-R-FRy} = \sum_{Age=18-19}^{50-54} \sum_{i=1day-3months}^{>=5years} DR_{vCJD-R-FR(age)y,i} \quad \text{(IV.C.1.c-27)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Defy}$- Number of recovered plasma donors potentially infected with vCJD in year y and deferred by current policy

Current FDA guidance (FDA 2002) recommends deferral of individuals who have history of travel to countries in Europe (other than the UK or France) since 1980 for an accumulated stay of 5 years or more from donating blood. The number of potential vCJD-infected recovered plasma donors who meet current deferral criteria (FDA 2002) was calculated by the equation:

$$DR_{vCJD-R-EU-Defy} = \sum_{Age=18-19}^{50-54} DR_{vCJD-R-EU(age)y,i>=5years} \quad \text{(IV.C.1.c-28)}$$

**Variable:** $DR_{vCJDS\text{-}EU\text{-}Resy}$ – The residual risk associated with recovered plasma donors potentially infected with vCJD that meet deferral criteria but because of limitations in the donor screening process and are not deferred by current policy; is represented by the equation:

$$DR_{vCJD-R-EU-Resy} = \sum_{Age=18-19}^{50-54} \sum_{i=1-30days}^{>=3-5years} DR_{vCJD-R-EU(age)y,i} \quad \text{(IV.C.1.c-29)}$$

## A-IV. C. 1. c. vi. Number of US recovered plasma donors who traveled to countries in Europe: Number potentially infected and vCJD agent is present in the blood

The most critical component of the model is the estimation of whether a plasma donation was collected from a vCJD infected donor that contained infectious vCJD agent in their blood (or was prionemic) at the time of donation. Based on data from animal studies, the model assumes that vCJD infectious individuals have infectious vCJD agent present in the blood during the last half of the incubation period. This portion of the model calculates the number of recovered plasma donors who may potentially contain infectious vCJD agent in their blood at the time of donation.

**Variable:** $Pr_{LH-y}$ -The probability an individual was prionemic in year 2002.

Variable: $DR_{vCJD\text{-}R\text{-}EUy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in European countries (other than France).

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residence in a country in Europe (other than the UK or France) and are in the last half incubation period of the disease (and has vCJD agent present in their blood).

$$DR_{vCJD-R-EU-LHy} = Binomial(DR_{vCJD-R-EUy}, \Pr_{LH-y}) \qquad \text{(IV.C.1.c-30)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}defy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in European countries (other than the UK or France) and met deferral criteria and were presumably deferred from donation .

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}def\text{-}LHy}$ - Total number of recovered plasma donors in the last half incubation period of the disease (and presumably has vCJD agent present in their blood) who met deferral criteria and were presumably deferred from donation.

$$DR_{vCJD-R-EU-Def-LHy} = Binomial(DR_{vCJD-R-EU-Defy}, \Pr_{LH-y}) \qquad \text{(IV.C.1.c-31)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Resy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during travel/residency in countries in Europe (other than the UK or France) and did not meet the deferral criteria and were likely not deferred .

**Variable:** $DR_{vCJD\text{-}R\text{-}EU\text{-}Res\text{-}LHy}$ - Total number of recovered plasma donors in the last half of the incubation period of the disease who did not met the deferral criteria and were likely not deferred from donation:

$$DR_{vCJD-R-EU-Res-LHy} = Binomial(DR_{vCJD-R-EU-Resy}, \Pr_{LH-y}) \quad \text{(IV.C.1.c-32)}$$

## A-IV. C. 1. d. Number of US plasma donors deployed by the military in the UK or other countries in Europe and potentially infected with vCJD

### A-IV. C. 1. d. i. Percentage of US plasma donors deployed at US military bases in the UK or other countries in Europe during the years 1980 through 1996

**Variable:** $Perc_{DR-DOD}$ - Percentage of US blood donors who were military residents in countries in Europe for ≥ 6 months from 1980 through 1996.

Assumption used in the model: Approximately 2% of US blood donors have been military residents in European countries between 1980-1996 (TSEAC 2002). There were no data for plasma donors, therefore, data for US blood donors was used to estimate the number of US donors stationed in US military facilities during the period 1980-1996

- The FDA model assumed that the same percentage of plasma donors have been in the military and deployed in European countries as blood donors.

### A-IV. C. 1. d. ii. Number of US plasma donors deployed by Military in the UK or other countries in Europe by year of deployment since 1980

### A-IV. C. 1. d. ii. a. Number of US Source Plasma donors stationed at US military facilities in the UK or countries in Europe during the period from 1980 -1996 by year of deployment

**Variable:** $y$ - Calendar year of deployment

**Variable:** $DODy$ - Number of US military residents, their family and dependents who resided on US military facilities in Europe by year from 1980 through 1996.

Assumption used in the model: The risk of BSE exposure and vCJD infection for donors previously deployed to US military facilities in the UK or countries in Europe after 1996 was assumed to be negligible, because it is assumed that most of the risk was associated with imported UK beef. Food chain controls put in place in the UK after 1996 were assumed to reduce the BSE exposure risk to negligible levels (TSEAC, 2002) and shipment of UK beef to US military facilities had stopped in 1996 or earlier.

**Variable:** *age* - age of donors in grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group (same variable used above in section A-IV.C.1.a.ii.)

$$Perc_{DR-DOD_y} = (DOD_y / \sum_{y=1980}^{1996} DOD_y) \times 100\% \quad \text{(IV.C.1.d-1)}$$

**Variable:** $DR_{S(age)}$ – Age of donors of Source Plasma (calculated in section A-IV.B. 1.)

**Variable:** $Perc_{DR\text{-}DOD}$ – Percentage of Source Plasma donors who have a history of military deployment in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{S\text{-}DOD(age)}$ - Estimated annual number of Source Plasma donors who have history of military deployment in the UK or Europe by age

**Assumption about variable:** We assumed 3% of Source Plasma donors have a history of military deployment and residence in the UK, France or other countries of Europe during any of the years from 1980-1996 and would have similar donation demographics and characteristics as whole blood donors.

The estimated annual number of Source Plasma donors who have a history of military deployment in Europe by age is represented by the equation:

$$DR_{S-DOD(age)} = DR_{S(age)} \times Perc_{DR-DOD} \qquad \text{(IV.C.1.d-2)}$$

**Variable:** $DR_{S\text{-}DOD(age)y}$ – Number estimated annual number of Source Plasma donors who have resided on military bases in Europe by age and deployment year

$$DR_{S-DOD(age)y} = DR_{S-DOD(age)} \times Perc_{DR-DODy} \qquad \text{(IV.C.1.d-3)}$$

## A-IV. C. 1. d. ii. b. Number of recovered plasma donors with a history of military deployment at US military facilities in the UK or other countries in Europe during the period 1980-1996 by year of deployment

**Variable:** *age* - Age of donors grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group. No data for the yearly distribution of deployment of military plasma donors in the UK, France or other countries in Europe was available. The age distribution of donors in the military was estimated from Department of Defense (DOD) data (2005). The donation rate for military staff was estimated using the blood donor survey data (TSEAC, 2002).

**Variable:** $DR_{R\text{-}(age)}$ - Annual number of recovered plasma donors by age

**Assumption used in the model:** We assumed 3% of recovered plasma donors were grouped by deployment year based on the yearly distribution of military deployment and residence in the UK or Europe during 1980-1996 .(DOD 2005).

$$DR_{R-DOD(age)y} = DR_{R-DOD(age)} \times Perc_{DR-DODy} \qquad \text{(IV.C.1.d-4)}$$

**Variable:** $DR_{R(age)}$ - Annual number of recovered plasma donors by age (calculated in section A-IV.B. 2.) grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of recovered plasma donors who have a history of military deployment in the UK and countries in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{R\text{-}DOD(age)}$ - Estimated annual number of recovered plasma donors who have a history of military deployment in the UK and countries in Europe by age.

Assumption used in the model: The same percentage of recovered plasma donors have a history of military deployment as blood donors.

The estimated annual number of recovered plasma donors who have history of military deployment in the UK and countries in Europe by age is represented by the equation:

$$DR_{R\text{-}DOD(age)} = DR_{R(age)} \times Perc_{DR\text{-}DOD} \quad \text{(IV.C.1.d-5)}$$

**Assumption used in the model:** The annual distribution of US military service members and their dependents residing on US military bases in Europe represents the yearly distribution of deployment in the UK and countries in Europe of US military recovered plasma donors

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \quad \text{(IV.C.1.d-6)}$$

## A-IV. C. 1. d. iii. Adjustment of the Relative Risk for the proportional variation in the BSE exposure risk in the UK and the military deployment duration per specific year during the period from 1980 - 1996

**Variable:** $R_{Base}$ - The cumulative vCJD risk of individual military personnel who were deployed and resided in the UK or Europe for the entire period from 1980 through 1996: it is assumed that the accumulated risk is equal to that of a UK resident and is assumed to be 1 (or equal to the UK vCJD prevalence) for this period.

**Assumption used in the model:** The cumulative risk of US military residents in Europe from 1980 through 1996 is 0.35. This estimate is based on the assumption that approximately 35% of the beef consumed by military personnel in Europe between 1980-1996 was imported from the UK (FDA 2002).

**Variable:** $y$ – Year of deployment (same variable used above in A-IV. C. 1. d. ii. a.)

**Variable:** $y_{epi}$ – The specific year of BSE epidemic in the UK.

Assumption about variable: The BSE epidemic in the UK was assumed to have started in 1980 and at first was not detected. The first cases of BSE were reported in 1986.

**Variable:** $BSE_{UKy}$ - Number of diagnosed BSE cases in the UK by year from 1980 through 1996

**Data used in the model:** Data are from the World organization for animal health (OIE 2005). Data were not collected for individual years prior to 1997. A total of 446 cases of BSE were reported by European countries (other than the UK) during the time period from 1980 through 1996 and were allocated to individual years by assuming the cases were increasing in a linear fashion by year.

**Variable:** $R_{Basey}$ - Proportional vCJD risk for donors that resided on US military bases in Europe in a specific year

**Assumptions used in the model:**

- The vCJD risk for US military facilities in Europe was present from 1980 through 1996. There was negligible vCJD risk after 1996 – the model assumed the major source of vCJD risk for US military bases in Europe was associated with imported UK beef. When food chain controls were implemented in the UK in 1996 – the model assumed the risk to be negligible.
- It was assumed that the vCJD risk was additive and can be prorated on a yearly base.
- The vCJD risk in a specific year was assumed to be proportional to the reported BSE cases in the UK in that specific year.

Proportional vCJD risk in the US military bases in a specific year was calculated by the equation:

$$R_{Basey} = R_{Base} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.d-7)}$$

**Variable:** $R_{DR\text{-}DODy}$ - Risk of individual military personnel who lived in Europe for a period of two years starting from deployment year y, and assumes the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The model assumed an average of two consecutive years of deployment:

$$R_{DR-DODy} = R_{Basey} + R_{Base(y+1)} \qquad \text{(IV.C.1.d-8)}$$

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (calculated in A-IV. C. 1.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}DOD(age)y}$ - Probability of infection for individual US plasma donor of a specific age group that lived on or near a US military bases in Europe starting from year y with duration of two years

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$\Pr_{vCJD-DR-DOD(age)y} = \Pr_{vCJD-UK(age)} \times R_{DR-DODy} \qquad \text{(IV.C.1.d-9)}$$

## A-IV. C. 1. d. iv. Number of all US plasma donors potentially infected with vCJD during residence at a US military base in the UK or other countries in Europe from 1980 to 1996

The section estimates the number of Source and recovered plasma donors with a history of deployment in the UK or countries in Europe during the period from 1980 through 1996 and sums the number of vCJD cases that may have vCJD agent in their blood (or be prionemic) at the time of donation from each pathway to derive the total number of all donors potentially infected with vCJD that may have vCJD agent in their blood (or be prionemic).

## A-IV. C. 1. d. iv. a. Potential number of US Source Plasma donors with a history of military deployment in the UK or other countries in Europe from 1980 to 1996 potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD(age)y}$ - Potential number of Source Plasma donors infected during residency on US military facilities in the UK or Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD\text{-}S\text{-}DOD(age)y} = Binomial(DR_{S\text{-}DOD(age)y}, Pr_{vCJD\text{-}DR\text{-}DOD(age)y}) \qquad \text{(IV.C.1.d-10)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD}$ - Potential number total of Source Plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the expression:

$$DR_{vCJD\text{-}S\text{-}DOD} = \sum_{y=1980}^{1996} \sum_{age=18-19}^{70-74} DR_{vCJD\text{-}S\text{-}DOD(age)y} \qquad \text{(IV.C.1.d-11)}$$

## A-IV. C. 1. d. iv. b. Potential number of Source Plasma donors with a history of deployment to a US military facility in the UK or other countries of Europe from 1980 to 1996 in the last half incubation period of the disease

This section estimates the number of Source Plasma donors that may potentially be infected with vCJD who may have vCJD agent in their blood (or be prionemic) at the time of donation.

**Variable:** $y$-The calendar year in which a plasma donor was deployed to a US military base in Europe.

**Assumption used in the model:** This risk assessment assesses the vCJD risk for pdFVIII product made in 2002 but it is assumed that the potential vCJD risk is similar to the present day risk in 2006.

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection/travel and the year 2002 when the plasma was collected.

**Variable:** $Pr\text{-}LH_y$- Probability that the vCJD disease is in the last half of the incubation period of the disease, if infected in year y and the individual has infectious vCJD agent present in their blood and plasma (or was prionemic).

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected is represented by the expression:

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.C.1.d-12)}$$

For an individual to be prionemic in 2002, the remaining period of time since infection up to 2002 ($I_{Inf-2002y}$) should be equal to or less than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially or the time between events. In this case it would be the time from infection to the time until the appearance of clinical disease (incubation period of the disease). The distribution is defined by two parameters: one that produces the shape of the curve; and a second generates the scale for the distribution, which in this case is represented by the mean incubation period of 14 years.

**Variable:** $Pr_{LH-y}$ -The probability an individual was prionemic in year 2002-was calculated by using the cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

**Variable:** $DR_{vCJD\text{-}S\text{-}DODy}$- Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment on European bases (calculated in A-IV. C. 1. d. v. a.).

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD\text{-}LHy}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment to US military bases in European countries and in the last half incubation period of the disease.

$$DR_{vCJD-S-DOD-LHy} = Binomial(DR_{vCJD-S-DODy}, \Pr_{LH-y}) \qquad \text{(IV.C.1.d-13)}$$

## A-IV. C. 1. d. iv. c. Number of US recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe during the period 1980-1996 and potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD(age)y}$ - Potential number of recovered plasma donors infected during deployment and residency on or near US military bases in Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD-R-DOD(age)y} = Binomial(DR_{R-DOD(age)y}, \Pr_{vCJD-DR-DOD(age)y}) \qquad \text{(IV.C.1.d-14)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD}$ - Potential number of recovered plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the equation:

$$DR_{vCJD-R-DOD} = \sum_{y=1980}^{1996} \sum_{age=15-19}^{70-74} DR_{vCJD-R-DOD(age)y} \qquad \text{(IV.C.1.d-15)}$$

## A-IV. C. 1. d. iv. d. Recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe: Potential number of donors in the last half of vCJD incubation period and vCJD agent is present in the blood

This portion of the model calculates the potential number of vCJD infected recovered plasma donors who are in the last half incubation period of the disease and presumably may contain vCJD agent in their blood (or are prionemic).

**Variable:** $Pr_{LH-y}$ -The probability a vCJD-infected donor had vCJD agent present in their blood and plasma at the time of donation (was prionemic) in the year 2002 (calculated in A-IV.C.1.d.v.b).

**Variable:** $DR_{vCJD\text{-}R\text{-}DODy}$- Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe (calculated in A-IV. C. 1. d. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD\text{-}LHy}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe and in the last half incubation period of the disease.

$$DR_{vCJD-R-DOD-LHy} = Binomial(DR_{vCJD-R-DODy}, \Pr_{LH-y}) \quad \text{(IV.C.1.d-16)}$$

## A-IV. C. 1. d. iv. e. Number of all vCJD infected plasma donors during deployment to a US military base in a country in Europe from 1980-1996

**Variable:** $DR_{vCJD\text{-}DOD}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996

$$DR_{vCJD-DOD} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD} \quad \text{(IV.C.1.d-17)}$$

**Variable:** $DR_{vCJD\text{-}DOD\text{-}Def}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

**Assumption used in the model:** Current policy defers individuals who have been deployed or resided on a US military base in Europe from 1980 to 1996 for a cumulative stay of 6 months or more. We assumed all US Department of Defense (DOD) deployments are 6 months or longer, and therefore, all individuals have a history of deployment to a US military base in Europe are deferred.

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \quad \text{(IV.C.1.d-18)}$$

## A-IV. C. 1. d. iv. f. Potential number of all plasma donors in the last half of vCJD incubation period

This portion of the model estimates the potential number of all vCJD infected plasma donors with a history of military service posted at a US military base in the UK or countries in Europe fron 1980-1996 who are in the last half incubation period of the disease and their blood and plasma presumably contain infectious vCJD agent (or are prionemic).

**Variable:** $DR_{vCJD\text{-}DOD\text{-}LH}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and are in the last half incubation period of the disease

$$DR_{vCJD-DOD-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD-LH} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD-LH} \qquad \text{(IV.C.1.d-19)}$$

**Variable:** $DR_{vCJD\text{-}DOD\text{-}Def}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \qquad \text{(IV.C.1.d-20)}$$

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## A-IV. C. 1. d. i. Percentage of US plasma donors deployed at US military bases in the UK or other countries in Europe during the years 1980 through 1996

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of US blood donors who were military residents in countries in Europe for $\geq$ 6 months from 1980 through 1996.

Assumption used in the model: Approximately 2% of US blood donors have been military residents in European countries between 1980-1996 (TSEAC 2002). There were no data for plasma donors, therefore, data for US blood donors was used to estimate the number of US donors stationed in US military facilities during the period 1980-1996

- The FDA model assumed that the same percentage of plasma donors have been in the military and deployed in European countries as blood donors.

## A-IV. C. 1. d. ii. Number of US plasma donors deployed by Military in the UK or other countries in Europe by year of deployment since 1980

## A-IV. C. 1. d. ii. a. Number of US Source Plasma donors stationed at US military facilities in the UK or countries in Europe during the period from 1980 -1996 by year of deployment

**Variable:** $y$ - Calendar year of deployment

**Variable:** ***DODy*** - Number of US military residents, their family and dependents who resided on US military facilities in Europe by year from 1980 through 1996.

**Assumption used in the model:** The risk of BSE exposure and vCJD infection for donors previously deployed to US military facilities in the UK or countries in Europe after 1996 was assumed to be negligible, because it is assumed that most of the risk was associated with imported UK beef. Food chain controls put in place in the UK after 1996 were assumed to reduce the BSE exposure risk to negligible levels (TSEAC, 2002) and shipment of UK beef to US military facilities had stopped in 1996 or earlier.

**Variable:** ***age*** - age of donors in grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group (same variable used above in section A-IV.C.1.a.ii.)

$$Perc_{DR-DODy} = (DOD_y / \sum_{y=1980}^{1996} DOD_y) \times 100\% \qquad \text{(IV.C.1.d-1)}$$

**Variable:** $DR_{S(age)}$ – Age of donors of Source Plasma (calculated in section A-IV.B. 1.)

**Variable:** $Perc_{DR\text{-}DOD}$ – Percentage of Source Plasma donors who have a history of military deployment in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{S\text{-}DOD(age)}$ - Estimated annual number of Source Plasma donors who have history of military deployment in the UK or Europe by age

**Assumption about variable:** We assumed 3% of Source Plasma donors have a history of military deployment and residence in the UK, France or other countries of Europe during any of the years from 1980-1996 and would have similar donation demographics and characteristics as whole blood donors.

The estimated annual number of Source Plasma donors who have a history of military deployment in Europe by age is represented by the equation:

$$DR_{S-DOD(age)} = DR_{S(age)} \times Perc_{DR-DOD} \qquad \text{(IV.C.1.d-2)}$$

**Variable:** $DR_{S\text{-}DOD(age)y}$ – Number estimated annual number of Source Plasma donors who have resided on military bases in Europe by age and deployment year

$$DR_{S-DOD(age)y} = DR_{S-DOD(age)} \times Perc_{DR-DODy} \qquad \text{(IV.C.1.d-3)}$$

## A-IV. C. 1. d. ii. b. Number of recovered plasma donors with a history of military deployment at US military facilities in the UK or other countries in Europe during the period 1980-1996 by year of deployment

**Variable:** *age* - Age of donors grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group. No data for the yearly distribution of deployment of military plasma donors in the UK, France or other countries in Europe was available. The age distribution of donors in the military was estimated from Department of Defense (DOD) data (2005). The donation rate for military staff was estimated using the blood donor survey data (TSEAC, 2002).

**Variable:** $DR_{R\text{-}(age)}$ - Annual number of recovered plasma donors by age

**Assumption used in the model:** We assumed 3% of recovered plasma donors were grouped by deployment year based on the yearly distribution of military deployment and residence in the UK or Europe during 1980-1996 .(DOD 2005).

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \qquad \text{(IV.C.1.d-4)}$$

**Variable:** $DR_{R(age)}$ - Annual number of recovered plasma donors by age (calculated in section A-IV.B. 2.) grouped by five-year increments (e.g., 20-24, etc.) and the 18-29 year old group

**Variable:** $Perc_{DR\text{-}DOD}$ - Percentage of recovered plasma donors who have a history of military deployment in the UK and countries in Europe since 1980 (calculated in section A-IV. C. 1. d. ii. a.)

**Variable:** $DR_{R\text{-}DOD(age)}$ - Estimated annual number of recovered plasma donors who have a history of military deployment in the UK and countries in Europe by age.

Assumption used in the model: The same percentage of recovered plasma donors have a history of military deployment as blood donors.

The estimated annual number of recovered plasma donors who have history of military deployment in the UK and countries in Europe by age is represented by the equation:

$$DR_{R\text{-}DOD(age)} = DR_{R(age)} \times Perc_{DR\text{-}DOD} \qquad \text{(IV.C.1.d-5)}$$

**Assumption used in the model:** The annual distribution of US military service members and their dependents residing on US military bases in Europe represents the yearly distribution of deployment in the UK and countries in Europe of US military recovered plasma donors

$$DR_{R\text{-}DOD(age)y} = DR_{R\text{-}DOD(age)} \times Perc_{DR\text{-}DODy} \qquad \text{(IV.C.1.d-6)}$$

## A-IV. C. 1. d. iii. Adjustment of the Relative Risk for the proportional variation in the BSE exposure risk in the UK and the military deployment duration per specific year during the period from 1980 - 1996

**Variable:** $R_{Base}$ - The cumulative vCJD risk of individual military personnel who were deployed and resided in the UK or Europe for the entire period from 1980 through 1996: it is assumed that the accumulated risk is equal to that of a UK resident and is assumed to be 1 (or equal to the UK vCJD prevalence) for this period.

**Assumption used in the model:** The cumulative risk of US military residents in Europe from 1980 through 1996 is 0.35. This estimate is based on the assumption that approximately 35% of the beef consumed by military personnel in Europe between 1980-1996 was imported from the UK (FDA 2002).

**Variable:** $y$ – Year of deployment (same variable used above in A-IV. C. 1. d. ii. a.)

**Variable:** $y_{epi}$ – The specific year of BSE epidemic in the UK.

**Assumption about variable:** The BSE epidemic in the UK was assumed to have started in 1980 and at first was not detected. The first cases of BSE were reported in 1986.

**Variable:** $BSE_{UKy}$ - Number of diagnosed BSE cases in the UK by year from 1980 through 1996

**Data used in the model:** Data are from the World organization for animal health (OIE 2005). Data were not collected for individual years prior to 1997. A total of 446 cases of BSE were reported by European countries (other than the UK) during the time period from 1980 through 1996 and were allocated to individual years by assuming the cases were increasing in a linear fashion by year.

**Variable:** $R_{Basey}$ - Proportional vCJD risk for donors that resided on US military bases in Europe in a specific year

**Assumptions used in the model:**

- The vCJD risk for US military facilities in Europe was present from 1980 through 1996. There was negligible vCJD risk after 1996 – the model assumed the major source of vCJD risk for US military bases in Europe was associated with imported UK beef. When food chain controls were implemented in the UK in 1996 – the model assumed the risk to be negligible.
- It was assumed that the vCJD risk was additive and can be prorated on a yearly base.
- The vCJD risk in a specific year was assumed to be proportional to the reported BSE cases in the UK in that specific year.

Proportional vCJD risk in the US military bases in a specific year was calculated by the equation:

$$R_{Basey} = R_{Base} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.C.1.d-7)}$$

**Variable:** $R_{DR\text{-}DOD_y}$ - Risk of individual military personnel who lived in Europe for a period of two years starting from deployment year y, and assumes the cumulative risk of a UK individual from 1980 through 1996 is 1.

**Assumption used in the model:** The model assumed an average of two consecutive years of deployment:

$$R_{DR-DOD_y} = R_{Base_y} + R_{Base(y+1)} \qquad \text{(IV.C.1.d-8)}$$

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (calculated in A-IV. C. 1.).

**Variable:** $Pr_{vCJD\text{-}DR\text{-}DOD(age)_y}$ - Probability of infection for individual US plasma donor of a specific age group that lived on or near a US military bases in Europe starting from year y with duration of two years

**Assumption used in the model:** Probability of infection is proportional to the risk of exposure

$$Pr_{vCJD-DR-DOD(age)_y} = Pr_{vCJD-UK(age)} \times R_{DR-DOD_y} \qquad \text{(IV.C.1.d-9)}$$

## A-IV. C. 1. d. iv. Number of all US plasma donors potentially infected with vCJD during residence at a US military base in the UK or other countries in Europe from 1980 to 1996

The section estimates the number of Source and recovered plasma donors with a history of deployment in the UK or countries in Europe during the period from 1980 through 1996 and sums the number of vCJD cases that may have vCJD agent in their blood (or be prionemic) at the time of donation from each pathway to derive the total number of all donors potentially infected with vCJD that may have vCJD agent in their blood (or be prionemic).

## A-IV. C. 1. d. iv. a. Potential number of US Source Plasma donors with a history of military deployment in the UK or other countries in Europe from 1980 to 1996 potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD(age)_y}$ - Potential number of Source Plasma donors infected during residency on US military facilities in the UK or Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD-S-DOD(age)_y} = Binomial(DR_{S-DOD(age)_y}, Pr_{vCJD-DR-DOD(age)_y}) \qquad \text{(IV.C.1.d-10)}$$

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD}$ - Potential number total of Source Plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the expression:

$$DR_{vCJD-S-DOD} = \sum_{y=1980}^{1996} \sum_{age=18-19}^{70-74} DR_{vCJD-S-DOD(age)y} \qquad \text{(IV.C.1.d-11)}$$

## A-IV. C. 1. d. iv. b. Potential number of Source Plasma donors with a history of deployment to a US military facility in the UK or other countries of Europe from 1980 to 1996 in the last half incubation period of the disease

This section estimates the number of Source Plasma donors that may potentially be infected with vCJD who may have vCJD agent in their blood (or be prionemic) at the time of donation.

**Variable:** *y*-The calendar year in which a plasma donor was deployed to a US military base in Europe.

**Assumption used in the model:** This risk assessment assesses the vCJD risk for pdFVIII product made in 2002 but it is assumed that the potential vCJD risk is similar to the present day risk in 2006.

**Variable:** $T_{Inf\text{-}2002y}$-Time period between infection/travel and the year 2002 when the plasma was collected.

**Variable:** $Pr\text{-}LH_y$- Probability that the vCJD disease is in the last half of the incubation period of the disease, if infected in year y and the individual has infectious vCJD agent present in their blood and plasma (or was prionemic).

**Variable:** $T_{Inf\text{-}2002y}$ - Time period between infection/travel and 2002 when the plasma was collected is represented by the expression:

$$T_{Inf-2002\,y} = 2002 - y \qquad \text{(IV.C.1.d-12)}$$

For an individual to be prionemic in 2002, the remaining period of time since infection up to 2002 ($I_{Inf\text{-}2002y}$) should be equal to or less than the half of incubation period of the disease.

**Assumption used in the model:** The variability and uncertainty of the incubation period of vCJD is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent processes that occur sequentially or the time between events. In this case it would be the time from infection to the time until the appearance of clinical disease (incubation period of the disease). The distribution is defined by two parameters: one that produces the shape of the curve; and a second generates the scale for the distribution, which in this case is represented by the mean incubation period of 14 years.

**Variable:** $Pr_{LH\text{-}y}$ -The probability an individual was prionemic in year 2002-was calculated by using the cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD_y}$- Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment on European bases (calculated in A-IV. C. 1. d. v. a.).

**Variable:** $DR_{vCJD\text{-}S\text{-}DOD\text{-}LH_y}$ - Total number of Source Plasma donors potentially infected with vCJD in year y during military deployment to US military bases in European countries and in the last half incubation period of the disease.

$$DR_{vCJD\text{-}S\text{-}DOD\text{-}LH_y} = Binomial(DR_{vCJD\text{-}S\text{-}DOD_y}, \Pr_{LH\text{-}y}) \quad \text{(IV.C.1.d-13)}$$

## A-IV. C. 1. d. iv. c. Number of US recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe during the period 1980-1996 and potentially infected with vCJD

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD(age)y}$ - Potential number of recovered plasma donors infected during deployment and residency on or near US military bases in Europe from 1980-1996 by age, and year of deployment

$$DR_{vCJD\text{-}R\text{-}DOD(age)y} = Binomial(DR_{R\text{-}DOD(age)y}, \Pr_{vCJD\text{-}DR\text{-}DOD(age)y}) \quad \text{(IV.C.1.d-14)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD}$ - Potential number of recovered plasma donors infected during residency on US military bases in Europe from 1980-1996 is represented by the equation:

$$DR_{vCJD\text{-}R\text{-}DOD} = \sum_{y=1996}^{1996} \sum_{age=10-19}^{70-74} DR_{vCJD\text{-}R\text{-}DOD(age)y} \quad \text{(IV.C.1.d-15)}$$

## A-IV. C. 1. d. iv. d. Recovered plasma donors with a history of deployment to a US military base in the UK or other countries in Europe: Potential number of donors in the last half of vCJD incubation period and vCJD agent is present in the blood

This portion of the model calculates the potential number of vCJD infected recovered plasma donors who are in the last half incubation period of the disease and presumably may contain vCJD agent in their blood (or are prionemic).

**Variable:** $Pr_{LH\text{-}y}$ -The probability a vCJD-infected donor had vCJD agent present in their blood and plasma at the time of donation (was prionemic) in the year 2002 (calculated in A-IV.C.1.d.v.b).

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD_y}$- Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe (calculated in A-IV. C. 1. d. v. c.)

**Variable:** $DR_{vCJD\text{-}R\text{-}DOD\text{-}LH_y}$ - Total number of recovered plasma donors potentially infected with vCJD in year y during military deployment on or near bases in Europe and in the last half incubation period of the disease.



606

$$DR_{vCJD-R-DOD-LHy} = Binomial(DR_{vCJD-R-DODy}, \Pr_{LH-y}) \quad \text{(IV.C.1.d-16)}$$

## A-IV. C. 1. d. iv. e. Number of all vCJD infected plasma donors during deployment to a US military base in a country in Europe from 1980-1996

**Variable:** $DR_{vCJD-DOD}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996

$$DR_{vCJD-DOD} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD} \quad \text{(IV.C.1.d-17)}$$

**Variable:** $DR_{vCJD-DOD-Def}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

**Assumption used in the model:** Current policy defers individuals who have been deployed or resided on a US military base in Europe from 1980 to 1996 for a cumulative stay of 6 months or more. We assumed all US Department of Defense (DOD) deployments are 6 months or longer, and therefore, all individuals have a history of deployment to a US military base in Europe are deferred.

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \quad \text{(IV.C.1.d-18)}$$

## A-IV. C. 1. d. iv. f. Potential number of all plasma donors in the last half of vCJD incubation period and have vCJD agent present in the blood

This portion of the model estimates the potential number of all vCJD infected plasma donors with a history of military service posted at a US military base in the UK or countries in Europe fron 1980-1996 who are in the last half incubation period of the disease and their blood and plasma presumably contain infectious vCJD agent (or are prionemic).

**Variable:** $DR_{vCJD-DOD-LH}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and are in the last half incubation period of the disease

$$DR_{vCJD-DOD-LH} = \sum_{y=1980}^{1996} DR_{vCJD-S-DOD-LH} + \sum_{y=1980}^{1996} DR_{vCJD-R-DOD-LH} \quad \text{(IV.C.1.d-19)}$$

**Variable:** $DR_{vCJD-DOD-Def}$ - Potential number of total plasma donors infected during residence on US military bases in Europe from 1980-1996 and meet deferral criteria

$$DR_{vCJD-DOD-Def} = DR_{vCJD-DOD} \quad \text{(IV.C.1.d-20)}$$

**Variable:** $DR_{Tot}$ - (calculated in section A-IV.B.3.) Annual number of plasma donors

**Variable:** $DR_S$ - (calculated in section A-IV.B.1.) Annual number of Source Plasma donors

**Variable:** $DR_R$ - (calculated in section A-IV.B.2.) Annual number of recovered plasma donors

**Variable:** $Perc_{DR\text{-}Eurob}$ - Percentage of blood donors who were Euroblood recipients

**Assumption used in the model:** 1.2% plasma donors were Euroblood recipients

**Variable:** $DR_{Eurob}$ - Annual number of plasma donors who were Euroblood recipients

$$DR_{Eurob} = DR_{Tot} \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-1)}$$

**Variable:** $DR_{S\text{-}Eurob}$ - Annual number of Source Plasma donors who were Euroblood recipients

**Assumption used in the model:** We assumed 1.2% of US Source Plasma donors were Euroblood recipients

$$DR_{S-Eurob} = DR_S \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-2)}$$

**Variable:** $DR_{R\text{-}Eurob}$ - Annual number of recovered plasma donors who were Euroblood recipients – represented by the equation:

$$DR_{R-Eurob} = DR_R \times Perc_{DR-Eurob} \quad \text{(IV.C.1.e-3)}$$

## A-IV. C. 1. e. Annual number of US plasma donors who have been Euroblood recipients

## A-IV. C. 1. e. i. Annual number of US plasma donors who have been Euroblood recipients

## A-IV. C. 1. e. ii. Annual number of potential vCJD-infected Euroblood donors and estimated annual units of Euroblood potentially containing vCJD agent

This section of the model estimates the quantity of Euroblood units predicted to have been transfused into US plasma donors in a one year period, the number of European donors involved, the number of possible vCJD infected European Euroblood donors, and the total quantity of vCJD infected units given by the Euroblood donors.

**Variable:** $DNBl_{UK(age)}$ - Blood donations in the UK by age of donors.

**Data used in the model:** Information about UK blood donors was provided by CDSC (2005).

**Variable:** $Perc_{DN\text{-}UK(age)}$ - Percentage distribution of the blood donations in the UK by donor age, and is represented by equation:

$$Perc_{DN\text{-}UK(age)} = DN_{BI\text{-}UK(age)} / \sum_{age=18}^{>65} DN_{BI\text{-}UK(age)} \quad \text{(IV.C.1.e-5)}$$

**Variable:** $EUBL_{(age)}$ - Units of Euroblood that were donated by a specific age group of European donors and transfused into US plasma donors

Total units of Euroblood received by US plasma donors of one year period is allocated by age of European donors based on the age distribution of UK blood donors

Assumption used in the model: We assumed the age distribution for Euroblood donors is the same as UK blood donors

$$EUBL_{(age)} = EUBL_{Tot} \times Perc_{DN\text{-}UK(age)} \quad \text{(IV.C.1.e-6)}$$

**Variable:** $DR_{EUBL(age)}$ – The number of European donors were grouped by age in five-year increments (e.g., 20-24 yrs, etc) and the 18-19 yr old group that have contributed Euroblood that may have been transfused into US plasma donors per one year period

Each European donor may give multiple donations in a single year; however the chance of more than one donation from same donor being shipped to the US and used by US plasma donors is expected to be small.

Assumption used in the model: Each unit of Euroblood received by US plasma donors of one year-period came from different European donors and is expressed by the equation:

$$DR_{EUBL(age)} = EUBL_{(age)} \quad \text{(IV.C.1.e-7)}$$

The probability of infection of individual European blood donors is calculated below based on the probability of infection per individual UK resident and the relative risk of a resident in a country elsewhere in Europe (other than France) compared to the risk of a UK resident.

**Variable:** $R_{EU}$ – The cumulative risk of an individual European resident from 1980 till the present; assumes that the cumulative risk of a UK individual from 1980 through 1996 is 1 (same variable as used in (A-IV.C.1.c.iii)

**Variable:** $Pr_{vCJD\text{-}UK(age)}$ - Probability of infection for individual UK resident of a specific age group (same variable as used in (A-IV.C.1. c. iv.).

**Variable:** $Pr_{vCJD\text{-}EU(age)}$-Probability of infection for an individual European resident of a specific age group.

Assumption used in the model: Probability of infection is proportional to the risk of exposure:

$$Pr_{vCJD-EU(age)} = Pr_{vCJD-UK(age)} \times R_{EU} \quad \text{(IV.C.1.e-7)}$$

**Variable:** $DR_{vCJD\text{-}EUBL(age)}$-Annual number of infected European donors who contributed Euroblood that was transfused into US plasma donors during a one-year period by age group.

Number of infected Euroblood donors among each age group was estimated using a binomial distribution function with the estimated total number of donors in the subgroup ($DR_{EUBL(age)}$ estimated in section A-IV.C. 1.e. ii) and the probability of infection for the individual of this age group of Euroblood donors ($Pr_{vCJD\text{-}DR\text{-}EUBL(age)}$ estimated in this section A-IV. C. 1. e. ii) as parameters of the distribution.

$$DR_{vCJD-EUBL(age)} = Bionomial(DR_{EULL(age)}, Pr_{vCJD-EU(age)}) \quad \text{(IV.C.1.e-8)}$$

**Variable:** $EUBL_{vCJD}$ - Total units of infected Euroblood received by US plasma donors of one year period

Potential infected European donor may give multiple donations in a single year, however the chance of more than one donation being from a single infected European donor being shipped to the US, and used by US plasma donors is expected to be small.

Assumption about variable: One infected European donor produces one unit of infected Euroblood.

$$EUBL_{vCJD} = \sum_{age=16-19}^{>65} DR_{vCJD-EUBL(age)} \quad \text{(IV.C.1.e-9)}$$

## A-IV. C. 1. e. iii. Annual number of plasma donors potentially infected with vCJD via transfusion with Euroblood

## A-IV. C. 1. e. iii. a. Annual potential number of vCJD infected plasma donors

**Variable:** $Pr_{vCJD\text{-}EUBL}$ - Probability a single unit of Euroblood contains vCJD agent

$$Pr_{vCJD-EUBL} = EUBL_{vCJD} / EUBL_{Tot} \quad \text{(IV.C.1.e-10)}$$

**Variable:** $Pr_{vCJD\text{-}EUBL\text{-}Recip}$ - Probability a Euroblood recipient is infected with vCJD

**Assumption used in the model:** We assumed that a Euroblood recipient is likely infected if he/she receives one unit or more of blood blood from a vCJD-infected donor.

$$Pr_{vCJD-EUBL-recip} = 1 - Binomdist(0, EUBL_{avg}, Pr_{vCJD-EUBL}, false) \quad \text{(IV.C.1.e-11)}$$

APPENDIX A

The Excel function Binomdist (n, N, p, false) calculates the probability of n "success" outcomes in a test, if the outcome of each trial of the test is either "success" or "failure", the probability of getting the outcome of "success" in an individual trial throughout the test is a constant $p$, and the number of trials in the test is $N$. In the problem we addressed here the outcomes are the probability of a donation being from a Euroblood recipient that receives a donation that is either infected or not infected. In equation IV.C.1.e-11, $Binomdist(0, EUBL_{avg}, Pr_{vCJD\text{-}EUBL}, false)$ calculated the probability of a recipient receiving no infected unit ($n$=0), under the condition that average number of units received by a recipient is $EUBL_{avg}$ ($N$=$EUBL_{avg}$) and probability a single unit of Euroblood being infected is $Pr_{vCJD\text{-}EUBL}$ ($p$=$Pr_{vCJD\text{-}EUBL}$).

The number of Source and recovered plasma donors infected through transfusion with Euroblood was estimated using a binomial distribution function with the estimated total number of source and recovered plasma donors who have received Euroblood ($DR_{S\text{-}EUBL}$ and $DR_{R\text{-}EUBL}$ estimated in section A-IV.C. 1.e. ii. ) and the probability of infection for the individual Euroblood recipient ($Pr_{vCJD\text{-}EUBL\text{-}receip}$ estimated in section A-IV. C. 1. e. iii) as parameters of the distribution. The total estimated number of potential plasma donors infected due to transfusion using Euroblood is the sum of potential infected source and recovered plasma donors.

**Variable:** $DR_{vCJD\text{-}S\text{-}EUBL}$ - Annual Number of Source Plasma donors being infected due to transfusion with a Euroblood unit:

$$DR_{vCJD-S-EUBL} = Binomial(DR_{S-EUBL}, \Pr_{vCJD-EUBL-recip}) \quad \text{(IV.C.1.e-12)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}EUBL}$ - Annual number of recovered plasma donors possibly infected via transfusion with a unit of Euroblood

$$DR_{vCJD-R-EUBL} = Binomial(DR_{R-EUBL}, \Pr_{vCJD-EUBL-recip}) \quad \text{(IV.C.1.e-13)}$$

**Variable:** $DR_{vCJD\text{-}EUBL}$ - Annual number of all plasma donors possibly infected through transfusion with a unit of Euroblood

$$DR_{vCJD-EUBL} = DR_{vCJD-S-EUBL} + DR_{vCJD-R-EUBL} \quad \text{(IV.C.1.e-14)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Def}$ - Annual number of plasma donors possibly infected through transfusion with a unit of Euroblood and meet deferral criteria and presumably deferred from donation.

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Res}$ - Annual number of plasma donors potentially infected via transfusion with a unit of Euroblood and does not meet deferral criteria and likely not deferred from donation.

Under current blood donation policies recipients of Euroblood are not deferred and represented by the expressions:

$$DR_{vCJD-EUBL-Def} = 0 \quad \text{(IV.C.1.e-15)}$$

$$DR_{vCJD-EUBL-Res} = DR_{vCJD-EUBL} \quad \text{(IV.C.1.e-16)}$$

## A-IV. C. 1. e. iii. b. Annual number of plasma donors that received Euroblood and are potentially infected and vCJD agent is present in the blood

**Assumption used in the model:** All infected Euroblood recipients have vCJD agent present in their blood (prionemic)

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Pn}$ - Annual number of plasma donors infected via transfusion using Euroblood and are prionemic in 2002

$$DR_{vCJD-EUBL-Pn} = DR_{vCJD-EUBL} \quad \text{(IV.C.1.e-17)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Def\text{-}Pn}$- Annual number of plasma donors infected via transfusion using Euroblood, and may have vCJD agent present in their blood and plasma (prionemic) in 2002 and meet deferral criteria

$$DR_{vCJD-EUBL-Def-Pn} = DR_{vCJD-EUBL-Def} \quad \text{(IV.C.1.e-18)}$$

**Variable:** $DR_{vCJD\text{-}EUBL\text{-}Res\text{-}Pn}$- Annual number of plasma donors who are possibly infected with vCJD via transfusion with Euroblood, that had vCJD agent present in their blood and plasma (prionemic) in 2002 and did not meet deferral criteria (likely not deferred)

$$DR_{vCJD-EUBL-Res-Pn} = DR_{vCJD-EUBL-Res} \quad \text{(IV.C.1.e-19)}$$

## A-IV. C. 1. f. Total number all plasma donors who may potentially be infected with vCJD through all sources of exposure and vCJD agent is present in the blood

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn}$ - Estimated total annual number of Source Plasma donors infected with vCJD wh potentially had the agent present in blood and plasma (prionemic) in 2002:

$$DR_{vCJD-S-Pn} = DR_{vCJD-S-UK-LH} + DR_{vCJD-S-FR-HL} + DR_{vCJD-S-DOD-LH} + DR_{vCJD-S-EUBL-Pn} \quad \text{(IV.C.1.f-1)}$$

**Variable:** $DR_{vCJD\text{-}R\text{-}Pn}$ - Estimated total annual number of recovered plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-R-Pn} = DR_{vCJD-R-UK-LH} + DR_{vCJD-R-FR-HL} + DR_{vCJD-R-EU-LH} + DR_{vCJD-R-DOD-LH} + DR_{vCJD-R-EUBL-Pn}$$

(IV.C.1.f-2)

**Variable:** $DR_{vCJD\text{-}Pn}$ - Total annual number of all US plasma donors potentially infected with vCJD with the agent present in blood and plasma (prionemic) in 2002

$$DR_{vCJD-Pn} = DR_{vCJD-S-Pn} + DR_{vCJD-R-Pn} \quad \text{(IV.C.1.f-3)}$$

## A-IV. C. 2. Annual number of all US plasma donors potentially infected with vCJD agent present in the blood and who may not be deferred by questionnaire screening

### A-IV. C. 2. a Annual number of US Source Plasma donors potentially infected and vCJD agent is present in the blood that may not be deferred by questionnaire screening

**Variable:** $Eff_{Def}$ - Effectiveness of US donor deferral policy

**Assumption about variable:** Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Variable:** $DR_{vCJD\text{-}S\text{-}Def\text{-}Pn}$ - Estimated annual number of Source Plasma donors potentially infected with and having vCJD agent present in blood and plasma (prionemic) that are deferred by current policy

$$DR_{vCJD\text{-}S\text{-}Def\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-1)}$$

**Assumption about variable:** This population includes the potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have long-term travel history to the UK (≥3 mo), and France (≥ 5 yrs); and a history of military deployment (or military dependent, etc.) in Europe from 1980 – 1996.

**Variable:** $DR_{vCJD\text{-}S\text{-}Res}$ - Estimated annual number of Source Plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that have short term travel history (UK (<3 mo), and France and/or Europe (< 5 yrs); and not deferred by deferral policy.

$$DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-2)}$$

**Assumption about variable:** This population includes the potential Source Plasma donors with vCJD with agent present in blood and plasma (prionemic) that have short-term travel history to the UK (< 3 mo), France (< 5 yrs), and a history of receiving Euroblood.

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn\text{-}NR}$ - Annual total number of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) and were not removed by deferral screening

**Assumption used in model:** This includes potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) who meet deferral criteria but for a variety of reasons are not deferred.

$$DR_{vCJD\text{-}S\text{-}Pn\text{-}NR} = DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}Def\text{-}Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-3)}$$

## A-IV. C. 2. b Annual number of US recovered plasma donors potentially infected and vCJD agent is present in the plasma that may not be deferred by questionnaire screening

**Variable:** $Eff_{Def}$ - Effectiveness of donor deferral policy

**Assumption about variable:** Based on advice from the TSEAC at the October 31, 2005 meeting, the FDA model assumed 85-99% of potential vCJD infected donors would have been deferred just prior to donation.

**Variable:** $DR_{vCJD\text{-}R\text{-}Def\text{-}Pn}$ - Estimated annual number of potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) who are deferred by current policy

$$DR_{vCJD\text{-}R\text{-}Def\text{-}Pn} = DR_{vCJD\text{-}R\text{-}UK\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}EU\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}R\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-4)}$$

**Assumption about variable:** Model includes potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) that have long term travel history to the UK (≥3 mo), France (≥ 5 yrs), and Europe (≥5 yrs); and history of military deployment, military dependent or related travel or residence in Europe.

There is a possibility that some individuals that traveled to the UK, France, and other countries in Europe since 1980 stayed for periods of time that were shorter than the deferral period, were exposed to BSE agent, and were infected with vCJD. These individuals represent a source of residual risk – or the remaining risk after interventions (in this case donor deferral policies) are applied. The section below addresses the calculation of residual risk for non-deferred at risk donors that traveled for periods of time that were shorter than recommended guidelines.

**Variable:** $DR_{vCJD\text{-}R\text{-}Res}$ - Annual number of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have short term travel history and are not covered by and deferred by deferral policy.

$$DR_{vCJD\text{-}S\text{-}Res\text{-}Pn} = DR_{vCJD\text{-}S\text{-}UK\text{-}Res\text{-}Pn} + DR_{vCJD\text{-}S\text{-}FR\text{-}Def\text{-}Pn} + DR_{vCJD\text{-}S\text{-}DOD\text{-}Pn} \quad \text{(IV.C.2-2)}$$

**Assumption about variable:** Estimation of the possible vCJD residual risk includes potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) that have short-term travel history to the UK (< 3 mo), France (< 5 yrs), history of travel to Europe and history of receiving Euroblood.

**Variable:** $DR_{vCJD\text{-}S\text{-}Pn\text{-}NR}$ - Annual total of potential Source Plasma donors with vCJD agent present in blood and plasma (prionemic) and were not removed by deferral screening

**Assumption used in model:** This includes all potential donors with vCJD agent present in blood and plasma (prionemic) that do not meet deferral criteria and who meet deferral criteria but wrongly not deferred.

$$DR_{vCJD-S-Pn-NR} = DR_{vCJD-S-Res-Pn} + DR_{vCJD-S-Def-Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-3)}$$

**Variable:** $DR_{vCJD-R-Pn-NR}$ - Annual total of potential recovered plasma donors with vCJD agent present in blood and plasma (prionemic) not removed by deferral screening

$$DR_{vCJD-R-Pn-NR} = DR_{vCJD-R-Res-Pn} + DR_{vCJD-R-Def-Pn} \times (1 - Eff_{Def}) \quad \text{(IV.C.2-4)}$$

## A-IV. C. 2. c Total number of all US plasma donors potentially infected and vCJD agent is present in the plasma but may not be deferred by questionnaire screening

The total number of all US plasma donors potentially infected with vCJD with agent present in blood and plasma (prionemic) that may not be deferred by questionnaire screening was determined by summing the estimates generated for both Source and recovered plasma donors that may not be deferred by current screening procedures, and is described by the equation:

$$DR_{vCJD-Pn-NR} = DR_{vCJD-S-Pn-NR} + DR_{vCJD-R-Pn-NR} \quad \text{(IV.C.2-5)}$$

## A-IV. C. 3. Total number of all US plasma donations potentially containing vCJD agent

**Variable:** $Freq_{DN-S}$ –Average frequency of donations from a single Source Plasma donor who contributes Source Plasma for FVIII manufacture (times/year)

**Variable:** $DN_{vCJD-S}$ - Annual number of potential vCJD donations of Source Plasma

$$DN_{vCJD-S} = DR_{vCJD-S-Pn-NR} \times Freq_{DN-S} \quad \text{(IV.C.2-6)}$$

Assumption used in the model: The average frequency of donations from a single Source Plasma donor who contributes Source Plasma for a FVIII plasma pool used in manufacturing ranges from [redacted] and most likely to be [redacted] based on data from pdFVIII manufacturers.

**Variable:** $Freq_{DN-R}$ –The average frequency of donations from a single recovered plasma donor who contribute plasma for pdFVIII manufacture (times/year) is 1.

**Variable:** $DN_{vCJD-R}$ - Annual number of potential vCJD donations of recovered plasma

$$DN_{vCJD-R} = DR_{vCJD-R-Pn-NR} \times Freq_{DN-R} \quad \text{(IV.C.2-7)}$$

Assumption used in the model: The average frequency of donations from a single blood donor that contributes recovered plasma for pdFVIII manufacture is 1.

## A-IV. C. 4. Probability that a US plasma donation potentially contained vCJD agent among all donations

**Variable: $Pr(DN_{vCJD-S})$** - Probability a donation of Source Plasma contained vCJD agent

**Variable: $DN_s$**-Annual units of Source Plasma used to make pdFVIII (calculated in A-IV. B. 1)

$$Pr(DN_{vCJD-S}) = DN_{vCJD-S} / DN_S \qquad \text{(IV.C.4-1)}$$

**Variable: $Pr(DN_{vCJD-R})$** - Probability a donation of recovered plasma contained vCJD agent.

**Variable: $DN_R$**-Annual number of units of recovered plasma used to make pdFVIII from plasma collected in the US (calculated in A-IV. B. 2).

$$Pr(DN_{vCJD-R}) = DN_{vCJD-R} / DN_R \qquad \text{(IV.C.4-2)}$$

## A-IV. C. 5. Probability of a Source or recovered plasma pool potentially containing a vCJD donation(s)

### A-IV. C. 5. a. Probability that a plasma pool may contain a specific number of vCJD donations

Assumption used in the model: Consistent with manufacturing practices in which commingling of Source and recovered plasma is uncommon, the risk assessment considered plasma pools to consist entirely of only Source Plasma donations or only recovered plasma donations.

**Variable: $n_{vCJD-DN-pool}$** - Designated number of vCJD donors in a single plasma pool.

Assumption used in the model: The number of vCJD donations in a single vCJD pool could be 0, 1, 2, 3 or 4, but because of the low prevalence of vCJD most of the time there would be 0 vCJD donations in a pool

**Variable: $DR_{pool-S}$** -Size of Source Plasma pool (donors/pool)

Data used in the model: Based on information provided to the FDA by pdFVIII manufacturers, an individual Source Plasma pool may contain 6,000 to 60,000 donors. A statistical distribution representing the variation in the size (number of donations per pool) of plasma pools used in the manufacture of pdFVIII was generated by combining information on pool size with information on the percentage of market share for several individual pdFVIII products.

**Variable: $Pr(n_{vCJD-DR-pool-S})$**- Probability a Source Plasma pool containing $n_{vCJD-DN-pool}$ infected donations ($n_{vCJD-DR-pool}$ =0, 1, 2, 4)-are determined by density frequency of $DR_{pool-S}$ at $X=n_{vCJD-DR-pool}$

**Variable:** $DN_{pool\text{-}R}$ -Size of recovered plasma pool (donors/pool)

**Data used in the model:** Based on information from manufacturers, individual Source Plasma pool may contain 6,000 to 60,000 donors.

**Variable:** $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$- Probability a Source Plasma pool containing $n_{vCJD\text{-}DN\text{-}pool}$ infected donations ($n_{vCJD\text{-}DR\text{-}pool}$=0, 1, 2, 4)-are determined by density frequency of $DR_{pool\text{-}S}$ at $X=n_{vCJD\text{-}DR\text{-}pool}$

**Variable:** $DN_{pool\text{-}R}$ -Size of recovered plasma pool (donors/pool)

**Data used in the model:** Based on information provided to the FDA by pdFVIII manufacturers, an individual recovered plasma pool may contain 150,000 to 360,000 donors. As with Source Plasma pools described above, a statistical distribution representing the variation in the size (number of donations per pool) of plasma pools used in the manufacture of pdFVIII was generated.

**Assumptions used in the model:** The size of recovered plasma pools was represented in the model by using a uniform distribution ranging from 150,000 to 360,000 donations per pool (Figure 4-3) – representing the range of pool sizes used by manufacturers of pdFVIII. The uniform distribution provided the best fit for the range of possible recovered plasma pool sizes that may be used in the US to manufacture pdFVIII.

**Variable:** $Pr(n_{vCJD\text{-}DN\text{-}pool\text{-}R})$-Probability a recovered plasma pool containing $n_{vCJD\text{-}DN\text{-}pool}$ infected donations ($n_{vCJD\text{-}DN\text{-}pool}$=0, 1, 2, 4)-are determined by density frequency of $DR_{pool\text{-}R}$ at $X=n_{vCJD\text{-}DR\text{-}pool}$

## A-IV. C. 5. b. Probability a plasma pool may potentially contain a vCJD donation(s)

**Variable:** $Pr(vCJD\text{-}pool_S)$-Probability of a Source Plasma pool containing one or more vCJD donations

$$\Pr(vCJD\_pool_S) = 1 - \Pr(n_{vCJD-DN-poolS} = 0) \quad \text{(IV.C.5-1)}$$

**Variable:** $Pr(vCJD\text{-}pool_R)$-Probability of a recovered plasma pool containing one or more vCJD donations

$$\Pr(vCJD\_pool_R) = 1 - \Pr(n_{vCJD-DN-pool_R} = 0) \quad \text{(IV.C.5-2)}$$

**Variable:** $Pr(vCJD\text{-}pool)$-The Probability that a plasma pool (including Source and recovered plasma pools) contained one or more vCJD donations- The distribution for pool size (or number of donations per pool) incorporated information on pool size.

**Variable:** $Perc_S$ – Percentage of Source Plasma pools used to manufacture pdFVIII in the US

**Variable:** $Perc_R$ – Percentage of recovered plasma pools used to manufacture pdFVIII in the US

**Assumption used in the model:** Estimates suggest that approximately [redacted] of pdFVIII products were made from Source Plasma, and [redacted] were made from recovered plasma.

The probability of either a vCJD donation being present in a Source Plasma pool is represented by the variable $Pr(vCJD\text{-}pool_S$ and the probability of a vCJD donation being present in a recovered plasma pool is represented by the variable, $Pr(vCJD\text{-}pool_R$, which was calculated in the section above. A discrete distribution (X1, X2; p1, p2) represents two discrete values for the probabilities that a pool may contain a vCJD donation, X1(or $Pr(vCJD\text{-}pool_S)$ and X2 (or $Pr(vCJD\text{-}pool_R)$ and the associated probabilities of each value occurring with the probabilities, p1 and p2, respectively. Based on the assumptions above that Source Plasma pools are used more frequently in the manufacture of pdFVIII and, on average contain fewer donations, the probability of a Source Plasma pool containing vCJD agent is different from the probability a recovered plasma pool containing vCJD agent. Overall probability of a single plasma pool (including source and recovered plasma pool) containing vCJD agent is a probability weight based on the percentages of the two types of plasma pools [redacted] for Source and [redacted] for recovered plasma pools) used to make pdFVIII. *Pr(vCJD-pool)* is sampled from $Pr(vCJD\text{-}pool_S$ and $Pr(vCJD\text{-}pool_R$ using the discrete distribution:

$$\Pr(vCJD-pools) = Discrete(Pr(vCJD\text{-}pool\text{-}s), Pr(vCJD\text{-}pool\text{-}R); Perc_S, Perc_R)) \quad \text{(IV.C.5-3)}$$

**or**

$$\Pr(vCJD-pools) = Discrete(Pr(vCJD\text{-}pool\text{-}s), Pr(vCJD\text{-}pool\text{-}R); [redacted])$$

## A-IV. D. Annual total number of all plasma pools and number of plasma pools potentially containing a vCJD donation that are used to make pdFVIII in the US

### A-IV. D.1. Annual amount of pdFVIII distributed in the US

**Variable:** $IU_{FVIII}$ -Annual number of all units of human pdFVIII manufactured and distributed in the US .

**Data used in the model:** Based on data provided to FDA from manufacturers, a total of [redacted] million units of pdFVIII was made and distributed in the US.

**Variable:** $Perc_s$ – Represents the percentage of pdFVIII assumed in the model to be made from Source Plasma (same as variable used in A-IV. C. 5. b.)

**Variable:** $Perc_R$ – Represents the percentage of pdFVIII assumed in the model to be made from recovered plasma (same as variable used in A-IV. C. 5. b.)

**Variable:** $IU_{FVIII\text{-}S}$ –The total annual number of units of pdFVIII made from Source Plasma and is represented by the equation:

$$IU_{FVIII-S} = IU_{FVIII} \times Perc_s \qquad \text{(IV.D.1-1)}$$

**Variable:** $IU_{FVIII\text{-}R}$ -The total annual number of units of pdFVIII made from recovered plasma and is represented by the equation:

$$IU_{FVIII-R} = IU_{FVIII} \times Perc_R \qquad \text{(IV.D.1-2)}$$

## A-IV. D. 2. Annual total number of all plasma pools used to make pdFVIII and plasma pools with vCJD agent

The total number of plasma pools used to make pdFVIII in the US each year can be back-calculated from the total number of units of human plasma-derived pdFVIII distributed in the US each year. Based on information described in earlier sections, it was assumed that approximately [redacted] of the total pdFVIII supply distributed annually in the US is manufactured from Source Plasma and [redacted] from recovered plasma pools. Information on pool size (number of donors), average number of donations per donor, size of individual recovered plasma donations (200 mls) and Source Plasma donations (700 mls) were used to first determine the amount of plasma present in a pool. Then, data on the average yield of pdFVIII per liter of plasma (187 IU), was used to calculate the total number of Source and recovered plasma pools and the results were summed to determine the total number of plasma pools used to manufacture pdFVIII in the US each year. The total number (or percentage) of plasma pools potentially containing vCJD agent was determined in the model based on pool size and the probability that a pool contained a vCJD agent.

### A-IV. D. 2. a. Amount plasma per pool

**Variable:** $DN_{V\text{-}S}$ – Volume of single unit Source Plasma (ml).

**Variable:** $DR_{pool\text{-}S}$ – Number donors per Source Plasma pool (same variable as used in A-IV.C. 5).

**Variable:** $Freq_{DN\text{-}S}$ –Average frequency of donations from a single Source Plasma donor that contribute Source Plasma for pdFVIII manufacture (same variable as used in A-IV.C. 3).

**Variable:** $V_{pool\text{-}S}$ – Volume of a Source Plasma pool (ml).

$$V_{pool-S} = DR_{pool-S} \times Freq_{DN-S} \times DN_{V-S} \quad \text{(IV. D. 2-1)}$$

**Variable:** $DN_{V\text{-}R}$ – Volume of single unit recovered plasma (ml).

**Variable:** $DR_{pool\text{-}R}$ – Number donors per recovered plasma pool (same variable as used in A-IV.C. 5. a.)

**Variable:** $Freq_{DN\text{-}S}$ –Average frequency of donations from a single recovered plasma donor that contribute recovered plasma for pdFVIII manufacture (same variable as used in A-IV.C. 3)

**Variable:** $V_{pool\text{-}S}$ – Volume of a recovered plasma pool (ml)

$$V_{pool-R} = DR_{pool-R} \times Freq_{DN-R} \times DN_{V-R} \text{ (IV. D. 2-2)}$$

## A-IV. D. 2. b. Annual number of plasma pools used to manufacture pdFVIII in the United States

**Variable:** $IU_{FVIII\text{-}S}$ -Annual units of pdFVIII made from Source Plasma (calculated in A-IV. D. 1)

**Variable:** $Y_{avg}$ –Average yield of pdFVIII (IU/L plasma)

**Assumption used in the model:** Based on the data provided by WFH (1998) and FDA-CBER (2003) we assumed average yield of pdFVIII (including high purity and intermediated purity pdFVIII) being 187 IU per liter plasma.

The total number of Source Plasma pools and recovered plasma pools used each year in manufacturing US pdFVIII are calculated separately in the model. Estimates from each type of pool are then summed to get a total value for all pools.

**Variable:** $Pool_S$–Annual number Source Plasma pool used to make pdFVIII

$$Pool_S = Round((IU_S / Y_{avg}) / (V_{pool-S} / 1000)) \qquad \text{(IV. D. 2-3)}$$

**Variable:** $IU_{FVIII\text{-}R}$ -Annual units of pdFVIII made from recovered plasma (calculated in A-IV. D. 1.).

**Variable:** $Pool_R$–Annual number of recovered plasma pools used to make pdFVIII

$$Pool_R = Round((IU_R / Y_{avg}) / (V_{pool-R} / 1000)) \qquad \text{(IV. D. 2-4)}$$

Finally, the number of possible Source and recovered plasma pools are summed to generate the total number of plasma pools used in the manufacture of pdFVIII in the US.

**Variable: Pool**–Annual total number of plasma pool used to make pdFVIII

$$Pool = Pool_S + Pool_R \qquad \text{(IV. D. 2-5)}$$

## A-IV. D. 2. c. Annual number vCJD plasma pools used to manufacture pdFVIII in the United States

Annual number of vCJD pools is expected to be low because the US vCJD prevalence, even among donors that traveled to the UK, France or other countries in Europe since 1980, is likely very low and presumably varies from year to year. A binomial distribution (n, p) is used to reflect the variation in the number of vCJD pools present in a single year. A binomial distribution is usually used when the number of positive observations (p) or in this case the number of vCJD containing pools is very low compared to the total number of pools (n).

**Variable:** $Pr(vCJD\text{-}pool_S)$- Probability of a Source Plasma pool containing vCJD agent

**Variable:** $Pool_{vCJD\text{-}S}$ – Annual number Source Plasma pools that contain vCJD agent used to make pdFVIII

$$Pool_{vCJD-S} = Binomial(Pool_S, \Pr(vCJD - pool_S)) \quad \text{(IV. D. 2-6)}$$

**Variable:** $Pr(vCJD\text{-}pool_R)$- Probability of a recovered plasma pool containing vCJD agent

**Variable:** $Pool_{vCJD\text{-}R}$ – Annual number of recovered plasma pools that contain vCJD agent used to make pdFVIII

$$Pool_{vCJD-R} = Binomial(Pool_R, \Pr(vCJD - pool_R)) \quad \text{(IV. D. 2-7)}$$

**Variable:** $Pool_{vCJD}$ – Annual total plasma pools that contains vCJD agent used to make pdFVIII

$$Pool_{vCJD} = Pool_{vCJD-S} + Pool_{vCJD-R} \quad \text{(IV. D. 2-8)}$$

## A-IV. D. 3. Percentage of pools potentially containing vCJD agent

**Variable:** $Perc_{vCJD\text{-}S\text{-}pool}$ -Percentage Source Plasma pools used to make pdFVIII that contains vCJD donations

$$Perc_{vCJD-S-pool} = (Pool_{vCJD-S} / Pool_S) \times 100\% \quad \text{(IV. D. 3-1)}$$

**Variable:** $Perc_{vCJD\text{-}R\text{-}pool}$ -Percentage recovered plasma pools used to make pdFVIII that contains vCJD donations

$$Perc_{vCJD-R-pool} = (Pool_{vCJD-R} / Pool_R) \times 100\% \quad \text{(IV. D. 3-2)}$$

**Variable:** $Perc_{vCJD\text{-}pool}$ –Overall percentage plasma pools used to make pdFVIII that contains vCJD donations

## A-IV. E. Module 2: Estimation of Quantity of vCJD agent in a plasma pool that contains a donation from a donor potentially infected with vCJD

### A-IV.E.1. Quantity of vCJD agent present in a donation of a specific donor potentially infected with vCJD

**Variable:** $I_{bl}$ – Represents the i.c. $ID_{50}$ present in the blood of individual infected donor ($ID_{50}$/ml) in the last half of the incubation period of vCJD.

**Assumption used in the model:** Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. $ID_{50}$ per ml, a 5th percentile of 2 i.c. $ID_{50}$ per ml, a median of 12 i.c. $ID_{50}$ per ml, a 95th percentile of 30 i.c. $ID_{50}$ per ml and a maximum of 1,000 i.c. $ID_{50}$ per ml. Attempts to identify vCJD infectivity titers in human blood have not been successful, but the assay sensitivity for vCJD *in vitro* and in animal models is limited (Bruce *et al* 2001 and Wadsworth *et al* 2001). Wadsworth *et al* estimated a limit of sensitivity of about 1,000 $ID_{50}$/ml by their assay meaning that infected blood containing less than 1,000 $ID_{50}$ would not have elicited infection or disease in their animal model, hence infectivity would not have been detected (Wadsworth, 2001).
**Variable:** $I_{pl\text{-}perc}$ – Percent (%) i.v. $ID_{50}$s associated with plasma

Studies in animal models have shown that greater than 50% of transmissible spongiform encephalopathy agent present in whole blood is associated with plasma. Experiments by Gregori *et al.* (2004) using a hamster – sheep scrapie model showed that approximately 58% of infectivity in whole blood is associated with plasma.

**Assumption used in the model:** The model assumes that 58% of infectivity is associated with plasma.

**Assumption used in the model:** Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

**Variable:** $DN_V$-Volume of one unit of plasma, depending on plasma type (same as $DN_{V\text{-}S}$ used in A-IV. D. 2 for Source Plasma, same as $DN_{V\text{-}R}$ used in A-IV. D. 2. for recovered plasma)

**Variable:** $I_{DN}$-Quantity of vCJD agent in one donation of infected plasma (i.v. $ID_{50}$/ml)

$$I_{DN} = I_{bl} \times DN_V \times I_{Pl-perc} \times A_{iv-ic} \qquad \text{(IV.E.1-1)}$$

## A-IV.E. 2. Quantity of vCJD agent in a plasma pool containing a donation from donor potentially infected with vCJD

**Variable:** $DN_{DR}$ - Number of donations from an infected plasma donor, which varies based on type of plasma donated.

**Assumption used in the model:** We assumed individual infected Source Plasma donor most likely give donations to a pool, with minimum of 1, maximum of 12 donations. Individual infected recovered plasma donors most likely give only one donation to a pool.

**Variable:** $I_{Pool}$- Initial infectivity in an infected plasma pool is represented by the equation:

$$I_{Pool} = I_{DN} \times DN_{DR} \qquad \text{(IV.E.2-1)}$$

## A-IV. F. Estimation of the potential quantity of vCJD agent in pdFVIII products manufactured from pool(s) potentially containing a vCJD donation

The FDA model employed three stratifications of clearance:

- $2-3$ $\log_{10}$
- $4-6$ $\log_{10}$
- $7-9$ $\log_{10}$

Each of these levels of clearance was modeled separately. Most of the results are presented for the 4-6 $\log_{10}$ reduction during manufacture processing in the risk characterization section (Section V.) of this risk assessment.

**Assumptions used in the model:** The model assumed there are potentially three levels of reduction that may be achieved: a lower level of reduction (a range of 2 - 3 $\log_{10}$)-represented by uniform distribution (2, 3), medium level of reduction (a range of 4-6 logs, most likely, 5 $\log_{10}$)-represented by triangular distribution (4, 5, 6) and higher level of reduction (a range of 7-9 $\log_{10}$, most likely, $\log_{10}$)-represented by triangular distribution (7, 8, 9).

**Variable:** $I_{Pool}$- Initial infectivity in a specific infected plasma pool (calculated in A-IV. E.2)

**Variable:** $R_{Log}$- Potential log reduction in infectivity during processing

**Variable:** $I_{Pool-Ap}$- Remaining infectivity in a specific infected plasma pool after processing

$$I_{Pool-Ap} = I_{Pool} / 10^{R_{Log}} \qquad \text{(IV.F-1)}$$

**Variable:** $DR_{Pool}$- Size of plasma pool (number of donors/pool).

**Assumption used in the model:** The size of the plasma pools used in manufacturing was assumed to vary from pool to pool. In this risk assessment model, two different general distributions were used to represent frequency distribution of sizes of Source and recovered plasma pool based on the data provided by pdFVIII manufacturers.

**Data used in the model:** Information for Source Plasma pool size was collected by the FDA from pdFVIII manufacturers. The size of Source Plasma pools ranged from 6,000 donors per pool to 60,000

donors per pool with mean of [redacted] donations per pool. The distribution was generated based on the pool size data provided by pdFVIII manufacturers and the market share of the products based on information supplied annually to the FDA by manufacturers. Manufacturers supplied FDA with information on the average number of donations from individuals in the pool.

**Data used in the model:** Information for recovered plasma pool sizes was collected by the FDA from pdFVIII manufacturers. The size of recovered plasma pool ranged from 150,000 to 360,000 donations per pool. The distribution was generated based on the pool size data provided by pdFVIII manufacturers and the market share of the products. Manufacturers supplied FDA with information on the average number of donations from individuals in the pool.

**Variable:** $DN_{DR\text{-}Avg}$-Average number of donations from individual donors in the pool

**Assumption used in the model:** Data on the average number of donations per donor per pool were provided by manufacturers. We assumed the average number donations from individual donors varied from pool to pool. For Source Plasma, it was assumed to range from [redacted] donations per donor, with a most likely of [redacted] donations per donor. For recovered plasma, it was assumed that the most likely number of donations per donor was only 1.

**Variable:** $DN_V$-Volume of one unit of plasma, depending on plasma type (for Source Plasma, same as $DN_{V\text{-}S}$ used in A-IV. D. 2, recovered plasma, same as $DN_{V\text{-}R}$ used in A-IV. D. 2.)

**Variable:** $Y_{FVIII}$-Yield of pdFVIII (IU/L plasma)

**Assumption used in the model:** Based on the data provided by the World Federation of Hemophilia (2004) we assumed pdFVIII yield varies from pool to pool with minimum of 120, most likely of 187 and maximum of 250 IU per liter plasma.

**Variable:** $I_{Iu}$- Quantity of infectivity in the pdFVIII product made from a specific infected pool (i.v. $ID_{50}$ per IU)

$$I_{IU} = (I_{Pool-Ap} / (DR_{Pool} \times DN_{DR-Avg} \times DN_V)) \times 1000 / Y_{FVIII} \quad \text{(IV. F-2)}$$

## A-IV. G. FVIII utilization by HA and vWD patients and potential exposure to the vCJD agent through use of human pdFVIII

### A-IV. G. 1. FVIII utilization and potential exposure to the vCJD agent through use of human plasma-derived FVIII by severe HA patients

This risk assessment provides outputs that estimate the annual exposure for several patient subpopulations with Severe HA disease for patients in the following clinical treatment groups:

- Prophylaxis – No inhibitor

- Prophylaxis - With inhibitor
- Prophylaxis - With inhibitor and immune tolerance
- Episodic – No inhibitor
- Episodic - With inhibitor

The study collected a total of 17,848 records, each record representing a single year of medical data for a single HA patient. The comprehensive study collected standardized information on patient demographics, clinical treatment and outcome data. Patient medical records were obtained from treatment sites including: hemophilia treatment centers (HTCs), hospitals, clinics, physician's offices, home-care agencies, nursing homes, prison infirmaries, and dispensers of factor concentrates. The data, abstracted from medical records, tabulated all recorded factor concentrate utilization prescribed by quantity, type, purpose (e.g., prophylaxis, treatment of acute bleeds, or immune tolerance therapy) and total quantity used per calendar year. Among all the records collected in the study from 1993-1998, 1,993 were from HA patients with severe disease that had been treated with human pdFVIII and the records were further grouped into five clinical treatment subcategories based on treatment regimen, including: prophylaxis, no inhibitor; prophylaxis,with inhibitor; prophylaxis, with inhibitor and immune tolerance; episodic, no inhibitor; and episodic, with inhibitor. Data from each of the five subpopulations were analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate initial descriptive statistics and distributions of pdFVIII usage by the HA patients. The data containing annual pdFVIII utilization information for patients in each of the five treatment groups were further analyzed using Best Fit software (Palisade Corp, New York) to generate a statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. Overall, the Generalized Beta distribution provided the most reasonable and consistent fit for the pdFVIII utilization data among all of the patient treatment groups. The Generalized Beta distributions were then used in the model to approximate the distribution of utilization of pdFVIII in each of the five HA patient subpopulations. FDA used the original patient data to not only generate statistical distributions for each patient treatment subpopulation. FDA also used the original data to identify the minimum and maximum dosages used by patients in each specific treatment subcategory and truncated each distribution using these values. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. We also provide a summary of the pdFVIII usage data from the CDC sponsored six state study, and also summarize the input Generalized Beta distributions generated with each subset of data in Table A-4.5.

**Table A-4.5. Annual usage of pdFVIII by individual HA patients with severe disease-data and input distribution**

| | | Original Data | | | Input distribution (Generalized Beta distribution) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment Regimen | Inhibitor Status | n | Mean | 95% CI | α | β | (min, max) | Mean | 95% CI |
| *Prophylaxis* | No Inhibitor | 578 | 164394 IU | (13574, 518781) | 1.5159 | 10.02 | (300, 1200000) | 157949 | (21242, 282316) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | With Inhibitor – No Immune Tolerance | 63 | 198781 | (7859, 937480 | 1:4640 | 6.2861 | (2000, 800000) | 190523 | (26856, 447639) |
| | With Inhibitor – With Immune Tolerance | 62 | 569707 | (14315, 3222471) | 0.8782 | 5.5081 | (100000, 2000000) | 558700 | (33235, 1592943) |
| *Episodic* | No Inhibitor | 948 | 90489 | (3001, 345416) | 0.9882 | 10.60 | (0, 1000000) | 85270 | (4833, 244658) |
| | With Inhibitor | 151 | 169710 | (4099, 835729) | 0.6950 | 3.6822 | (2200, 1000000) | 180458 | (5314, 488906) |

**Variable:** $IU_{Yr}$ - Annual usage of pdFVIII by individual HA patient of a specific clinical group (IU/yr, person)

**Variable:** $IU_{vial}$ - Vial size (IU/vial)

**Assumption used in the model:** We assumed there were equal numbers of vials for each of the four different package sizes (250, 500, 1000 and 1500 IU/vial) that are distributed in the US.

**Variable:** $Vial_{Tot}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

**Assumption used in the model:** We assumed individual patient uses pdFVIII products of the same package size throughout the whole year period of 2002 for which the model was run.

$$Vial_{Tot} = IU_{Yr} / IU_{Vial} \qquad \text{(IV.G. 1-1)}$$

**Variable:** $Pool$ - Annual number of plasma pool used to make pdFVIII (calculated in A-IV.D.2.b.)

**Variable:** $Pool_{vCJD}$ - Annual number of vCJD plasma pool used to make pdFVIII (calculated in A-IV.D.2.c.)

**Variable:** $Perc_{vCJD-vail}$ – Percentage pdFVIII vials containing vCJD agent

**Variable:** $Vial_{vCJD}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

$$Vial_{vCJD} = Vial_{Tot} \times Perc_{vCJD-vial} \qquad \text{(IV.G. 1-2)}$$

**Variable:** $I_{iu}$ - Quantity of infectivity in the pdFVIII product made from a specific infected pool (i.v. $ID_{50}$ per IU) (calculated in IV. F)

**Variable:** $I_{yr}$ - Annual exposure to vCJD through use of pdFVIII (i.v. $ID_{50}$/yr, person)

$$I_{yr} = \sum_{i=1}^{Vial_{vCJD}} I_{IU} \times IU_{Vial} \qquad \text{(IV.G. 1-3)}$$

## A-IV. G. 2. pdFVIII utilization and annual exposure of severe von Willebrand disease patients

The CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 did not include patients with vWD. We assumed that vWD patients with severe disease would largely use Humate P product only for factor replacement treatment. A search of records in the Hemophilia Surveillance System project data revealed a total of 58 records that indicated Humate P had been used, among which, 8 records indicates patients had developed inhibitor, which are considered uncommon among vWD patients and were excluded from analysis. Among the 58 records, 35 were from Adults (≥15 yrs of age) and 23 records were from young persons (<15 yrs of age). Records for each age group were further grouped by clinical treatment using either a prophylaxis or episodic treatment regimen. Data were initially analyzed individually using the statistical package "JMP" (SAS Institute, Cary, NC) to generate descriptive statistics and statistical distribution(s) for each patient treatment group that best reflected the variation in pdFVIII utilization. The Generalized Beta distribution was identified as the best fit to the pdFVIII utilization data (as determined by using the software Best Fit (Palisade Corp, NY) and was used as the input distribution for pdFVIII usage by individual vWD patients in the model. Graphical representations of the original data and the fitted Generalized Beta distributions are shown in Appendix C. Table A-4.6. summarizes pdFVIII usage data from CDC sponsored study and the input distribution generated based on the data. FDA used data in the CDC and six state Hemophilia Surveillance System project conducted from 1993-1998 to estimate FVIII utilization by all vWD patients. The data represent only a sample of all possible vWD patients with severe disease in the US. FDA estimated that there were approximately 250 patients in the US with Type 3 vWD. To calculate the total number of patients in each age group and treatment regimen group we adjusted the 58 patient population to equal a total of 250 patients by multiplying the patient population in each group by a factor of 4.3 (250/58 = ~4.3). The utilization data for patients in each treatment regimen in the sample population were used in the risk assessment model to generate outputs for the annual exposure to vCJD for all vWD for Adult (>15 yrs of age) and Young (≤15 yrs of age) persons in the US among clinical treatment groups of prophylaxis and episodic.

**Table A-4.6. Annual usage of pdFVIII by individual severe vWD patient -data and input distribution We need to update the information in this table – based on new calculations for a total of 58 cases (previously it was 50 cases)**

| | Original Input Data | | | | Input Distribution (Generalized Beta distribution) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Treatment Regimen | n | Percent of total population | Mean | 95% CI | α | β | (min, max) | Mean | 95% CI |
| Young (≤15 yrs of age) | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Prophylaxis* | 9 | 16% | 164193 | (9200, 504625) | 0.4523 | 0.9794 | (9200, 504625) | 165713 | (9346, 479457) |
| *Episodic* | 14 | 24% | 11122 | (1010, 41850) | 0.3900 | 1.1973 | (1010, 41850) | 11045 | (1013, 37543) |
| **Adult** (>15 yrs of age) | | | | | | | | | |
| Prophylaxis | 17 | 29% | 187538 | (15000, 772800) | 0.5741 | 1.9559 | (15000, 7728000) | 186880 | (15570, 606699) |
| Episodic | 18 | 31% | 845556 | (1000, 293800) | 0.5855 | 1.4097 | (1000, 293800) | 86923 | (1361, 260660) |

**Variable:** ***IUyr*** - Annual usage of pdFVIII by individual vWD patient of a specific clinical group (iu/yr, person)

**Variable:** $IU_{vial}$ - Vial size (IU/vial)

**Assumption used in the model:** We assumed that equal numbers of vials in each of three different package sizes (250, 500, 1000 IU/vial) are distributed on the market.

**Variable:** $Vial_{Tot}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

**Assumption used in the model:** We assumed individual patients used pdFVIII products of the same package size through out whole year period of 2002 for which the model was run.

$$Vial_{Tot} = IU_{yr} / IU_{Vial} \qquad \text{(IV.G. 2-1)}$$

**Variable:** ***Pool*** - Annual number of plasma pool used to make pdFVIII (calculated in A-IV. D .2.b.).

**Variable:** $Pool_{vCJD}$ - Annual number of vCJD plasma pool used to make pdFVIII (calculated in A-IV.D.2.c.)

**Variable:** $Perc_{vCJD\text{-}vail}$ – Percentage pdFVIII vials containing vCJD agent

**Variable:** $Vial_{vCJD}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

$$Vial_{vCJD} = Vial_{Tot} \times Perc_{vCJD\text{-}vial} \qquad \text{(IV.G. 2-2)}$$

**Variable:** $I_{iu}$ - Quantity of infectivity in the pdFVIII product made from a specific infected pool (iv. $ID_{50}$ per IU) (calculated in IV.F.)

**Variable:** $I_{yr}$- Annual exposure of individual vWD patients to vCJD through use of pdFVIII (i.v. $ID_{50}$/yr, person)

$$I_{yr} = \sum_{i=1}^{Vial_{vCJD}} I_{IU} \times IU_{Vial} \qquad \text{(IV.G. 2-3)}$$

APPENDIX

# DRAFT ISSUE SUMMARY
# TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES ADVISORY COMMITTEE (TSEAC) MEETING
**December 15, 2006**
**Silver Spring, Maryland**

**Topic II: Experimental Clearance of Transmissible Spongiform Encephalopathy (TSE) Infectivity in Plasma-derived Factor VIII (pdFVIII) Products**

Issue: FDA seeks the Committee's advice on whether a minimum TSE agent reduction factor, demonstrated in laboratory-based experimental models, would enhance vCJD safety of the products.

**Background and Rationale**

Although variant Creutzfeldt-Jakob (vCJD) transmission by plasma derivatives has not been reported, plasma from experimental animals has been shown to contain TSE infectivity, and human blood transfusions have probably transmitted vCJD in the UK. Results from the FDA risk assessment model for potential exposure to the vCJD agent through the use of U.S. licensed pdFVIII products indicate that a major factor in reducing the potential risk of such exposure is the amount of TSE agent clearance that occurs during manufacturing (Attachment 1). TSE clearance in plasma products has been studied by many manufacturers on a voluntary basis. On February 20, 2003, the TSEAC voted that FDA should consider labeling claims for TSE clearance when manufacturers have submitted clearance studies using viral validation-style methods and model TSE spiking agents. To date, FDA has approved labeling claims for TSE clearance for two IGIV products and an Antithrombin III product. These approvals were based upon viral validation-style studies using brain-derived spikes for input infectivity, and bioassays as a read-out. Various other studies of pdFVIII products that have been performed by manufacturers were reported to the TSEAC on September 18, 2006 (Attachment 2)(1).
At the September 18, 2006 meeting, the Committee discussed TSE clearance study methods (1) (Summary, Attachment 3). Most members thought that it would be premature to standardize TSE clearance studies. Exogenous ("spiking") models for TSE clearance were deemed more feasible than experiments using plasma of infected animals because of logistical constraints on the endogenous model.[1] The Committee agreed that exogenous clearance studies have limitations, particularly due to lack of certainty about the form of the TSE agent in blood. However, should an exogenous model demonstrate that a process step or series of steps achieves a relatively low $\log_{10}$ reduction of spiked TSE infectivity, a study using a large volume of endogenously infected plasma might still be useful to determine whether or not the low demonstrated level of exogenous model clearance still offered some potential benefit. In these scaled-down endogenous model clearance experiments, it would be especially important to test most if not all of the final product using animals and routes of inoculation known to be highly sensitive for detecting infectivity. Although the logs of infectivity removed will not be large compared with an exogenous spiking experiment, the relevance of the endogenous experiment to

[1] Constraints include low dynamic range of clearance that can be demonstrated, scale of the experiment, number of assay animals required, and lengthy incubation times.

the human situation might be greater. This is because the form of TSE infectivity in the blood of an infected animal is more likely to resemble that found in human blood, as compared to brain or spleen derived spiking material. Because of the very small amounts of agent likely to be present in endogenously TSE-infected blood, any failure of complete TSE clearance would be highly significant.

On September 18, 2006, the Committee was asked to comment whether a minimum TSE reduction factor, demonstrated using an exogenous model in scaled-down manufacturing experiments, might serve as an appropriate standard for demonstrating vCJD safety of pdFVIII products. The TSEAC was also asked to comment on what actions FDA might consider if such a minimum TSE reduction factor were not achieved. The discussion was deferred to the current meeting so that the Committee could respond in the context of recently completed FDA risk assessments.

**Discussion**

To determine a likely appropriate threshold level of TSE clearance for pdFVIII, two separate lines of evidence should be considered: the amount of clearance needed to assure that infectivity is removed (based on amount of starting infectivity in plasma), and the impact of clearance results on the pdFVIII risk assessment.

In the somewhat similar case of viral clearance validation studies, typical results accepted by FDA in support of label claims usually demonstrated at least 4 $\log_{10}$ of clearance by each of two mechanistically dissimilar (orthogonal) steps.[2] In the case of TSEs, the amount of infectivity in blood or plasma of experimentally infected animals has been estimated as 2-30 intracerebral *infectious units (i.c. IU)/mL (2-4). An IU is the quantity of infectivity associated with a 100%* probability of infection in recipients. An $ID_{50}$ is the amount of infectivity associated with a 50% probability of infection in recipients. Therefore 1 IU = 2 $ID_{50}$. The amount of infectivity in the blood of BSE-infected and scrapie-infected ruminants and in the blood of vCJD-infected persons is unknown. If vCJD infectivity levels in human blood are similar to those found in rodent blood or plasma, then effective clearance might necessitate a reduction of infectivity by at least 4 $\log_{10}$, plus an additional margin of safety. Calculations of pathogen reduction are based upon removal of the absolute amount of infectivity, rather than upon infectivity concentration. For example, a plasma unit of 800 ml that contained infectivity of 2-30 IU/ml would contain 1,600 - 24,000 IU (3.2 - 4.4 $\log_{10}$).[3] A precise margin of safety for TSE clearance studies is difficult to specify, given current limitations in test methodology and uncertainties about the maximum infectivity titers in blood of asymptomatic vCJD-infected donors. In viral studies, an additional margin of safety that assures clearance of at least 2-3 $\log_{10}$ more than the highest anticipated titers of the viral pathogen has often been considered prudent.

The pdFVIII risk assessment provides additional information about TSE clearance and risk of vCJD exposure. The risk assessment was performed using $\log_{10}$ clearances of 2-3, 4-6, and 7-9. The level of risk is highly impacted by the amount of clearance achieved in product manufacturing. For example, assuming a higher prevalence of vCJD based on the UK tissue survey, a patient with severe hemophilia A who has no inhibitors and is on episodic treatment with pdFVIII is estimated to have a potential mean annual risk of vCJD of 1 in 159 if exposed to

[2] Estimated maximum levels of viremia range from $10^4$ to $10^7$ for enveloped viruses e.g. HIV-1, HCV, and HBV (5), and from $10^5$ to $10^{10}$ for non-enveloped viruses, HAV(6) and B19 (7) virus.

[3] Plasma collection volumes recommended by FDA are 625-800 mL, depending upon the donor's weight (http://www.fda.gov/ber/bldmem/110492.pdf).

a product with 2-3 $\log_{10}$ vCJD reduction, 1 in 105,000 if exposed to a product with 4-6 $\log_{10}$ vCJD reduction, and 1 in 100 million if exposed to a product with 7-9 $\log_{10}$ vCJD reduction (Attachment 1). Assuming a lower prevalence of vCJD based on the number of cases that have occurred and are projected to occur in the UK, a patient with severe hemophilia A who has no inhibitors and is on episodic treatment with pdFVIII is estimated to have a potential mean annual risk of vCJD of 1 in 21,500 if exposed to a product with 2-3 $\log_{10}$ vCJD reduction, 1 in 9.4 million if exposed to a product with 4-6 $\log_{10}$ vCJD reduction, and 1 in 3.2 billion if exposed to a product with 7-9 $\log_{10}$ vCJD reduction (Attachment 1)

In spite of the limitations of clearance studies and the uncertainties of risk assessment, a scientifically-based opinion about meaningful clearance of infectivity would provide a useful interim target to assess pdFVIII safety. FDA is considering what level of clearance, demonstrated in a well-designed scaled-down study using an exogenous spiking model, might provide a sufficient assurance of safety with respect to TSEs.

## Questions for the Committee:

1. Based on available scientific knowledge, please discuss whether a minimum TSE agent reduction factor, demonstrated using an exogenous (spiking) model in scaled-down manufacturing experiments, would enhance vCJD safety of the products.

2. If the Committee identifies a minimum TSE reduction factor that would enhance vCJD safety what actions should FDA consider in cases when a licensed pdFVIII has a lower reduction factor:
   a. Labeling that would differentiate the lower TSE clearance products from the higher TSE clearance products;
   b. Recommending addition of TSE clearance steps to the manufacturing method;
   c. Performance of TSE clearance experiments using endogenous infectivity models;
   d. Any other actions?

References

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6. Bower, WA, et al. Duration of viremia in Hepatitis A virus infection. Journal of Infectious Diseases 182: 12-17, 2000.

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**Attachment 1 - Range of *Predicted Annual Mean Potential vCJD risk for pdFVIII per HA Patient* –** *at three levels of clearance: 7-9 $log_{10}$, 4-6 $log_{10}$, and 2-3 $log_{10}$ and at Higher Prevalence and Lower Prevalence estimates and at different treatment doses. (excerpted from table 5.3.A in the FDA' s Draft Quantitative Risk Assessment of vCJD Risk Potentially Associated with the Use of Human Plasma-Derived Factor VIII Manufactured Under United States (US) License From Plasma Collected in the US*

| | | | | 7 - 9 $Log_{10}$ Reduction | | 4 - 6 $Log_{10}$ Reduction | | 2 - 3 $Log_{10}$ Reduction | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Model Output for LOWER vCJD Case Prevalence estimate of ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) | Model Output for LOWER vCJD Case Prevalence estimate ~1.8 in 1,000,000 based on Clark and Ghani (2005) | Model Output for HIGHER vCJD Infection Prevalence based on estimate of 1 in 4,225 by Hilton *et al* (2004) |
| Treatment Regimen | Inhibitor Status | Est. Total Number patients in US | Mean quantity of product used per person per year ($5^{th}$ - $95^{th}$ )[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] | Mean potential vCJD risk per person per year[a] ($5^{th}$ - $95^{th}$ perc)[b] |
| *Prophylaxis* | No Inhibitor | 578 | 157949 IU (21242 , 382316 ) | 1 in 4.1 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 4 million (0-0)[c] | 1 in 54,000 (0- 1 in 12,000) | 1 in 15,000 (0-0)[c] | 1 in 82 (0 - 1 in 17) |
| | With Inhibitor – No Immune Tolerance | 63 | 190523 IU (26956 , 447639) | 1 in 3.5 billion (0-0)[c] | 1 in 40 million (0 - 1 in 8.8 million) | 1 in 4.8 million (0-0)[c] | 1 in 41,000 (0- 1 in9,000) | 1 in 12,000 (0-0)[c] | 1 in 65 (0 - 1 in 13 ) |
| | With Inhibitor – With Immune Tolerance | 62 | 558700 IU ( 33235, 1592943) | 1 in 551 million (0-0)[c] | 1 in 15 million (0 - 1 in 3.4 million) | 1 in 1.3 million (0-0)[c] | 1 in 15,000 (0- 1 in3,700) | 1 in 2,700 (0-0)[c] | 1 in 24 (0 - 1 in 3 ) |
| *Episodic* | No Inhibitor | 946 | 85270 IU ( 4633, 244656) | 1 in 3.2 billion (0-0)[c] | 1 in 100 million (0 - 1 in 24 million) | 1 in 9.4 million (0-0)[c] | 1 in 105,000 (0- 1 in 24,000) | 1 in 21,500 (0-0)[c] | 1 in 159 (0 - 1 in 34 ) |
| | With Inhibitor | 151 | 160458 IU (5314 , 488906 ) | 1 in 4 billion (0-0)[c] | 1 in 50 million (0 - 1 in 11 million) | 1 in 8 million (0-0)[c] | 1 in 23,000 (0- 1 in 12,000) | 1 in 23,000 (0-0)[c] | 1 in 73 (0 - 1 in 16) |

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] The $5^{th}$- $95^{th}$ perc (percentiles) are the minimum and maximum numbers that define the range constituting the 90% confidence interval. Accordingly, the mean risk estimates from the model should fall within this defined interval at least 90% of the time.

[c] For a $5^{th}$ and $95^{th}$ percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of pdFVIII recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a pdFVIII plasma pool would be rare and more than 90% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.



655

BENE2006-025

Attachment 2 – TSE Clearance Study Results for pdFVIII, presented by the Plasma Protein Therapeutics Association at the TSEAC meeting of 9/18/06 at http://www.fda.gov/ohrms/dockets/ac/06/slides/2006-4240S1_7_files/frame.htm.

PPTA
Plasma Protein Therapeutics Association

## Company A

| Step | MAB column | Q-Sepharose chromatography |
|---|---|---|
| Spike | Scrapie strain 263K | Scrapie strain 263K |
| Preparation | 10% brain homogenate | 10% brain homogenate |
| Prion detection / quantification method | - Hamster bioassay<br>- Western blot confirmation | - Hamster bioassay<br>- Western blot confirmation |
| No. of independent runs per spike preparation | one | one |
| Log reduction(s), $ID_{50}$ | 4.6 | 3.5 |

TOTAL REDUCTION: 8.1 $\log_{10} ID_{50}$

→ Product is licensed in the USA

PPTA
Plasma Protein Therapeutics Association

## Company B

| Step | 3.5 % PEG Precipitation | Heparin Affinity Chromatography* | Saline Precipitation and Final Filtrations | TOTAL |
|---|---|---|---|---|
| Spike | $PrP^{Sc}$ 263K Scrapie | $PrP^{Sc}$ 263K Scrapie | $PrP^{Sc}$ 263K Scrapie | |
| Preparations | 1) Microsomal fraction<br>2) Detergent treated preparation | 1) Brain homogenate<br>2) Detergent treated preparation | 1) Microsomal fraction<br>2) Detergent treated preparation | |
| Prion detection / quantification method | WB | WB | WB | |
| No. of independent runs per spike preparation | 2 | 2 | 2 | |
| Log reduction(s) | 3.21 – 3.43 | ≥3.44 – ≥3.45 | 2.08 – 2.47 | |
| Mean | 3.32 | ≥3.45 | 2.28 | ≥9.05 |

* Preliminary results

→ Product is licensed in the USA

PPTA
Plasma Protein Therapeutics Association

## Company D

| Steps | Subsequent Precipitation Steps | Precipitation Step Followed by Polishing Step and Sterile Filtration |
|---|---|---|
| Spike | 263K Scrapie | 263K Scrapie |
| Preparation | Microsomes // purified $PrP^{Sc}$ | Microsomes // purified $PrP^{Sc}$ |
| Prion detection/quantification method | CDI (conformation-dependent immunoassay) | CDI (conformation-dependent immunoassay) |
| No. of independent runs/spike preparation | 2 per spike preparation | 2 per spike preparation |
| **Log reduction(s), Mean** | **3.5 // 3.9** | **2.9 // 4.0** |

→ Product is licensed in the USA

PPTA
Plasma Protein Therapeutics Association

## Company E

| Steps | Adsorption, Precipitation, and Chromatography |
|---|---|
| Spike | 263K Scrapie |
| Preparation | Clarified Scrapie Brain Homogenate (cSBH) and Microsomal Fraction |
| Prion detection/quantification method | PK treatment, 0.5 log titration, and one-step Western blot |
| No. of independent runs/spike preparation | 1 per spike preparation |
| Log reduction(s) | 3.8 for cSBH spike, 3.7 for microsomal spike |
| **Mean** | **3.7 to 3.8** |
| Comments: Consistent results were also obtained from partially combined experiments. An additional step is under evaluation. | |

→ Product is licensed in the USA

Attachment 3 – Summary of Topic I, TSE meeting 9/18/06 (at http://www.fda.gov/ohrms/dockets/ac/06/minutes/2006-4240M-updated.pdf

**Abbreviated Summary**
**For the**
**TRANSMISSIBLE SPONGIFORM ENCEPHALOPATHIES**
**ADVISORY COMMITTEE MEETING**
**September 18 & 19, 2006**
**Gaithersburg, MD**
At: http://www.fda.gov/ohrms/dockets/ac/06/minutes/2006-4240M-updated.pdf

**Topic 1: Experimental Clearance of Transmissible Spongiform Encephalopathy Infectivity in Plasma-derived Factor VIII Products**
FDA asked the Committee to discuss whether standardized methods and assessment criteria are feasible and appropriate for determining clearance of TSE agents by the manufacturing processes for plasma-derived FVIII (pdFVIII) products.
Dr. Dorothy Scott introduced the topic summarizing TSE safety concerns, the importance of TSE clearance, upstream pdFVIII manufacturing processes, and methodological and logistical challenges of TSE clearance studies using exogenous spiking materials or endogenously infected blood. She also discussed the question of whether a minimum TSE agent reduction factor might serve as an appropriate standard for demonstrating vCJD safety, similar to analogous precedents from viral validation studies. Then Dr. Thomas Kreil, PPTA, discussed specific TSE clearance study challenges with regard to scale-down and conditioning. Dr. Kreil also presented data from industry-sponsored TSE clearance studies for pdFVIII.
**Questions for the Committee**

**1. a. Please comment on the feasibility and scientific value of adopting standardized exogenous (spiking) study methods to assess TSE clearance in manufacturing of pdFVIII including the following:**

**Optimal spiking material and its preparation from the standpoint of relevance to blood infectivity**

The committee discussed several possibilities, including TSE-infected brain-derived spiking materials, such as hamster 263K brain homogenate which is frequently used, is partially characterized with regard to partitioning during fractionation, and provides sufficiently high titers of infectivity and $PrP^{TSE}$ to allow demonstration of a broad range of clearance in studies. Spleen-derived spikes have lower titers, and there is no guarantee that they represent the physical form of TSE agent in blood better than do brain spikes. It was suggested that, since VLDL fractions of blood may preferentially contain TSE infectivity (based on data from Dr. Safar), such fractions might usefully represent endogenous infectivity. Committee members felt that current experiments might begin with brain homogenate preparations, and that more definitively blood-relevant spikes or endogenous infectivity needed further study. It was widely acknowledged that the physical form of TSE agents in endogenously infected blood must be better understood before the most relevant spiking materials can be selected.

**II) Selection of a TSE strain and animal model**

Several models were discussed (e.g., PrP-bovinized transgenic mice, sheep, and chimeric transgenic mice). Bovinized mice are very susceptible to infection with vCJD agent, and conventional RIII mice can be used to model vCJD as well. It was suggested that, in theory, TSE-infected sheep blood could be assayed with RIII mice, enabling titration of large amounts of plasma or product intermediates. Mice lacking the PrP GPI anchor were also suggested as a possible model, since their blood titers of infectivity have been very high (although it is not known whether the form of infectious TSE agent and its associations in such deficient mice would faithfully model more typical infections). Some members of the committee felt that the most relevant strains of TSE agent to be studied would be derived from humans with vCJD or cows with BSE.

**III) TSE immunoassays for $PrP^{TSE}$ and bioassays for infectivity**

Members commented that conformation-dependent immunoassay (CDI) or protein misfolding cyclic amplification (PMCA) technique showed preliminary promising results. However, the committee discussed the need to compare and carefully validate CDI, PMCA, and other binding assays with bioassays, and some members felt that infectivity still should be demonstrated by bioassay.

**IV) Identification of manufacturing processes that might alter TSE agent properties**

The Committee members commented that the manufacturing process itself is not standardized and varies from product to product and manufacturer to manufacturer so that developing a standard method for validation will require further consideration.
Overall, efforts at standardization were felt by some to be premature, since characteristics of endogenous infectivity are still not well understood, and therefore difficult to model; standardization might even impede research to address remaining challenges in TSE clearance studies. A second viewpoint was expressed, that some standardization now might be useful, because as better methods are discovered they are inevitably adopted.

**1. b. Please comment on the feasibility and scientific value of adopting standardized endogenous study methods to assess TSE clearance in manufacturing of pdFVIII.**

The Committee discussed the merits of various models including the use of transgenic mice (e.g., PrP-cervidized mice for CWD, PrP-bovinized mice for BSE, and PrP GPI-deficient mice) and sheep models of infectivity. Dr. Kreil warned that a potential limitation of endogenous infectivity studies is that animal plasma is known to have characteristics somewhat different from those of human plasma when fractionated, so that manufacturing processes might not be comparable and results with animal models not predictive of those with human plasma. While data were not presented to support or refute this contention, the committee agreed that it might pose an additional limitation of studies using endogenous TSE infectivity in animal plasma.

**2. Based on available scientific knowledge, please discuss whether a minimum TSE agent reduction factor, demonstrated using an exogenous (spiking) model in scaled-**

**down manufacturing experiments, might serve as an appropriate standard for demonstrating vCJD safety of the products.**
A detailed discussion of this question was postponed until the next meeting when risk assessment results will be discussed. One member reminded the Committee of the need for a clear definition for "log reduction" of infectivity, recognizing that the 50-percent infectious dose (ID50) is a continuous rather than a discrete variable and that estimated reductions to less than a single ID50 do not guarantee safety.

**3. Considering the outcome of the discussion on Item 2, in cases where a lower reduction factor is demonstrated for a pdFVIII, should FDA consider the following:**

**a. Labeling that would differentiate the lower clearance products from other products with sufficient TSE clearance;**

**b. Recommending addition of TSE clearance steps to the manufacturing method;**

**c. Performance of TSE clearance experiments using endogenous infectivity models;**

**d. Any other actions?**

This answer depends on the answer to the previous questions, thus definitive discussions were deferred until more information is available. In limited discussion, some members felt that labeling of a product as having less clearance might unfavorably dispose consumers or physicians against certain products even though no vCJD infection has ever been attributed to any plasma derivative. A member felt that the patient community might favor adding effective clearance steps to a manufacturing process but that labeling of products with low clearance values is not indicated now and would not be helpful.

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
|---|---|---|---|---|---|---|
| | | | | 2007. 1. 26 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | | 公表国 |
|---|---|---|---|---|
| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | HPA Press Statement. 2007 Jan 18. | 英国 |

**研究報告の概要**

○輸血関連変異型クロイツフェルト・ヤコブ病の4例目

変異型クロイツフェルトーヤコブ病(vCJD)の可能性のある新規発症例が、後にvCJDを発症した供血者に由来する輸血を受けた患者で診断された。これは英国におけるvCJD患者で輸血後感染の可能性があるものの4症例目となる。

最初の輸血関連vCJD症例の発症は2003年12月に確認された。輸血の6年半後に発症し、供血の3年半後にvCJDを発症した供血者由来の血液を輸血されていた。2例目は、供血の18ヶ月後にvCJDを発症した供血者からの赤血球を輸血された受血者に発生した。この患者はvCJDとは無関係な原因で輸血5年後に死亡した。3例目の患者は輸血後6年で発症し8年8ヶ月で死亡したが、供血者は供血後約20ヶ月でvCJDを発症していた。

新規の、4例目となる感染症例は輸血を受けた8年半後にvCJDの症状を来した。患者はプリオンタンパク遺伝子のコドン129にメチオニンホモ接合体が見られた。この患者はまだ生存している。供血者は供血の17ヶ月後にvCJDを発症していた。また、3例目の症例にもvCJDに汚染された血液を供血していた。

4症例全てが1996年から1999年の間に白血球除去をされていない赤血球製剤を輸血されていた。1999年10月から英国では全ての輸血用血液から白血球が除去されたが、vCJD伝播リスクの削減効果については不確定である。

4番目の症例は、輸血によるvCJD伝播のリスクへの関心を高めるが、多くは不明のままである。血液と血液製剤によるvCJD伝播リスク軽減のための現在の予防措置の重要性が強調される。血漿分画製剤と関連したvCJD症例は報告されていない。

英国でvCJDに汚染された血液を輸血された少数の生存者は、輸血による潜在的なvCJD曝露を通知されている。彼らは医療行為によるvCJD伝播リスクを軽減させる適切な予防策を求められ、専門家による神経学的な評価とアドバイスを受けている。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 英国で輸血による感染が疑われる変異型クロイツフェルト・ヤコブ病の4例目が報告され、当該患者に赤血球を提供した供血者は3例目の患者にも血液を提供していたとの報告である。 | 日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。 |

# News

Last updated: **19 January 2007**; Volume 1; No 3

- ***Fourth case of transfusion-associated variant-CJD infection***

## *Fourth case of transfusion-associated variant-CJD infection*

A new case of probable variant Creutzfeldt-Jakob disease (vCJD) has recently been diagnosed in a patient who received a blood transfusion from a donor who later developed vCJD [1]. This is the fourth case of probable transfusion transmission of vCJD infection in the United Kingdom (UK). Three of the four recipients developed symptoms of vCJD.

The first symptomatic case of vCJD disease associated with blood transfusion was identified in December 2003. This individual developed vCJD six and a half years after transfusion of red cells donated by an individual who developed symptoms of vCJD three and a half years after donation.

A second case of vCJD 'infection' was identified a few months later in a recipient of red cells from a donor who developed symptoms of vCJD 18 months after donation. This case died from causes unrelated to vCJD five years after transfusion. Post-mortem investigations found abnormal prion protein in the spleen and a cervical lymph node, but not in the brain, and no pathological features of vCJD were found.

A third case developed symptoms of vCJD 6 years and died eight years and eight months after receiving a transfusion of red blood cells from a donor who developed vCJD about 20 months after this blood was donated. These three cases have been published as case reports [2-4] and in findings of the ongoing collaborative study between the National Blood Services, the National CJD Surveillance Unit, and the Office for National Statistics to collect evidence about transmission of CJD or vCJD via the blood supply [5].

The new, and fourth, case of infection developed symptoms of vCJD eight and a half years after receiving a transfusion of red blood cells from a donor who developed vCJD about 17 months after this blood was donated [1]. The donor to this case also donated the vCJD-implicated blood transfused to the third case. As for all other reported clinical vCJD cases that have been tested for genotype, this patient is a methionine homozygote at codon 129 of the prion protein gene. The patient is still alive.

All four cases had received transfusions of non-leucodepleted red blood cells between 1996 and 1999. Since October 1999, leucocytes have been removed from all blood used for transfusion in the UK. The effect of leucodepletion on the reduction of the risk of transmission of vCJD from an infective donation is uncertain.

This fourth case of vCJD infection associated with blood transfusion further increases the level of concern about the risk of vCJD transmission between humans by blood transfusion, although much remains unknown. This reinforces the importance of the existing precautions that have been introduced to reduce the risk of transmission of vCJD infection by blood and blood products [6]. No cases of vCJD have been associated with fractionated plasma products. The small group of living recipients of vCJD-implicated blood transfusion in the UK have been informed of their potential exposure to vCJD by blood transfusion, asked to take certain precautions to reduce the risk of onward person-to-person transmission of vCJD during healthcare, and offered specialist neurological evaluation and advice.

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2007年2月19日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | | 研究報告の公表状況 | Journal of The Royal Society Interface DOI: 10.1098/RSIF.2007.0216 | 公表国<br>イギリス | |
| 販売名<br>（企業名） | ①ヘブスブリン（ベネシス）<br>②静注用ヘブスブリンーIH（ベネシス） | | | | | |

**研究報告の概要**

輸血を介したvCJDに感染したと疑われる3症例の発見によって、英国でヒトからヒトへの二次感染の流行が起きる可能性の懸念が高まった。英国保健省は、直ちにこの脅威に対応し、1980年以降に輸血を受けた人からのドネーションを禁止した。我々は、この文書で血液由来のvCJDの流行の大きさを探るために感度分析（sensitivity analysis：vCJD感染者1名を流行開始時点で感受性者集団に入れた時の感染者数の期待値）を行い、公衆衛生的介入の有効性について調査した。数学的モデルを、基本再生産感染者数（basic reproduction number）の表現と併せて開発した。感染した血液を介したvCJDの伝播を決める未知のパラメーターに対するモデルの予測の感度を、悲観的なモデルを使った仮定のもとで評価した。自己持続的流行（RO>1）が起こりうるなら流行（2080年まで）の大きさは900例以内であることを我々は見出した。しかし、そのような流行が起こるシナリオは、生物学的にありそうもないことが判った。楽観的な仮定では、公衆衛生への介入は上限を250例に減らし、生物学的に妥当なシナリオのみを考慮したときには更に小さくなった。我々の結果は、大規模な又は自己持続性流行に至るシナリオの可能性はあるが実現性は低く、公衆衛生的介入が有効であるという考えを支持している。

**使用上の注意記載状況・その他参考事項等**

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 輸血を介したvCJDのヒトからヒトへの二次感染の流行が起こる可能性について、大規模な又は自己持続性流行に至るシナリオの可能性はあるが実現性は低く、輸血を受けたヒトからのドネーション禁止措置等の公衆衛生的介入が有効であることを統計的に説明した報告である。<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

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# Is there the potential for an epidemic of variant Creutzfeldt–Jakob disease via blood transfusion in the UK?

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The discovery of three individuals suspected to have contracted variant Creutzfeldt–Jakob disease (vCJD) through blood transfusions has heightened concerns that a secondary epidemic via human-to-human transmission could occur in the UK. The Department of Health responded immediately to this threat by banning those who had received blood transfusions since 1980 from donating blood. In this paper, we conduct a sensitivity analysis to explore the potential size of a blood-borne vCJD epidemic and investigate the effectiveness of public health interventions. A mathematical model was developed together with an expression for the basic reproduction number ($R_0$). The sensitivity of model predictions to unknown parameters determining the transmission of vCJD via infected blood was assessed under pessimistic modelling assumptions. We found that the size of the epidemic (up until 2080) was bounded above by 900 cases, with self-sustaining epidemics ($R_0 > 1$) also possible; but the scenarios under which such epidemics could arise were found to be biologically implausible. Under optimistic assumptions, public health interventions reduced the upper bound to 250 and further still when only biologically plausible scenarios were considered. Our results support the belief that scenarios leading to large or self-sustaining epidemics are possible but unlikely, and that public health interventions were effective.

**Keywords: basic reproduction number; blood transfusion; Creutzfeldt–Jakob disease; epidemiology; mathematical model**

## 1. INTRODUCTION

The primary epidemic of variant Creutzfeldt–Jakob disease (vCJD) is believed to have been caused by the consumption of beef infected by bovine spongiform encephalopathy (BSE; Bruce *et al.* 1997; Hill *et al.* 1997; Scott *et al.* 1999). As of January 2007, there have been 158 deaths attributed to vCJD in the UK, but the annual incidence of clinical vCJD has continued to decline since peaking in 2000, with only five deaths recorded in 2006 (NCJDSU 2007). While the tailing off of the epidemic is reassuring from a public health perspective, recent results suggest it is too early to assert that the epidemic is dying out. A study of 13 000 tonsil and appendix samples was conducted to assess the extent of vCJD infection in the UK population (Hilton *et al.* 2004). The prevalence of infection was estimated to be far higher than previously thought, and poses the question as to why there have been only hundreds rather than thousands of clinical cases. One explanation is that a large proportion of those infected entered a 'carrier state' in which they may be infectious to others, but will not go on to develop clinical symptoms during their lifetime (Hill & Collinge 2003). The subclinical vCJD infection hypothesis is supported by data from experiments involving animal prion diseases (Asante *et al.* 2002) and by statistical modelling of the vCJD epidemic (Clarke & Ghani 2005).

Compelling evidence of human-to-human transmission of vCJD by blood transfusion first emerged in autumn 2003 when a patient who died of vCJD was found to have received blood from a blood donor who himself went on to die of vCJD (Llewelyn *et al.* 2004). Subsequently, a second such case was identified (HPA 2006) and a third recipient of blood from a vCJD case was found to show signs of vCJD infection after *post-mortem* (Peden *et al.* 2004). Together with the unexpectedly high vCJD prevalence discussed above, these discoveries have led to fears that a large-scale self-sustaining epidemic of vCJD could arise via blood transfusion with serious implications for public health (Farrugia *et al.* 2005; Dolan 2006; Hilton 2006; Ironside 2006).

In this paper, we explore the potential for a secondary, blood-borne epidemic of vCJD by performing a sensitivity analysis based on a mathematical

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transmission model. We use this analysis to gain insight into how the scale of the epidemic may vary according to different assumptions about its epidemiology. We assume that the two clinical cases described above certainly contracted vCJD via blood transfusion and thus ignore any uncertainty about their actual infection routes. The sensitivity analysis is based on a deterministic compartmental model for transmission through the human population by infected individuals donating blood which is subsequently used in blood transfusions. Some model parameters index population demographics and the UK blood supply, for which robust estimates based on data from the Census and the National Blood Service (NBS) are used. The remaining parameters index the epidemiological characteristics of vCJD for which the estimates are less robust. For the primary epidemic via bovine-to-human transmission, we use estimates based on a previous model of the primary epidemic which allows a carrier state; for parameters of the blood-borne transmission process where much is uncertain, we vary these parameters over their domain to quantify the variation in epidemic predictions and produce bounds on the extent of the epidemic. It should be understood that it is impossible to produce fully robust predictions of the secondary epidemic at the current time, and we do not claim to do so. Instead, we aim to describe the dynamics of the epidemic and explore its order of magnitude in worse-case scenarios *given current knowledge*.

This paper is arranged as follows. In §2, we introduce the data on which estimates of the human mortality and the UK blood supply are based. In §3, we introduce a mathematical model, present an expression for the basic reproduction number under this model, extend the model to allow for interventions and describe the design of the sensitivity analysis. The results of the sensitivity analysis are presented in §4 and discussed in §5.

## 2. DATA SOURCES

Information about the population size in each birth cohort and survivorship within these cohorts from all causes were taken from UK Census data. The vast majority of blood is used for red cell transfusions and all three individuals suspected of contracting vCJD via blood transfusion received red blood cells (Hewitt *et al.* 2006). Hence, we restrict our analyses to this transmission route.

Data on the rates and types of red cell transfusions were obtained from a prospective observational study over 28 days carried out in the North of England (Wells *et al.* 2002). Over this period, the use of 9848 units of red blood cells was recorded, of which approximately 41% was used in surgery, 52% in general medicine (including anaemia, haematology and gastrointestinal bleeds) and 6% in obstetrics and gynaecology. Data on the survivorship of patients receiving blood transfusions were obtained from a related study in which 2899 patients at a single centre transfused in June 1994 were followed for 5 years (Wallis *et al.* 2004). A total of 10 760 red blood cell units were used for transfusions over this period. The age distribution of those receiving transfusions is similar to that obtained in the earlier study (Wells *et al.* 2002). After 5 years of follow-up, 47% of the transfusion recipients remained alive.

Blood donation data were obtained from the NBS, which covers all donations in England and North Wales (Det Norske Veritas 2003). These figures show that, in 1996/1997, there were 1.9 million donors who donated 2.2 million units of usable blood. More recent figures from 2004/2005 showed there were fewer donors (1.6 million), but each donor donated more blood (2.1 million units of usable blood). However, these later figures were obtained following the introduction of leucodepletion and changes in the definitions of those allowed to donate blood. Hence, we use the earlier figures to enable consideration of epidemic predictions prior to interventions (which include leucodepletion). Blood donors are normally between 18 and 65 years of age. Donors are allowed to donate blood up to 3 times per year, but on average do so 1.2 times per year.

Age is potentially a critical factor determining vCJD spread via blood transfusion and must be included in the mathematical model. Epidemiological analysis of the primary epidemic found vCJD to depend strongly on age, with a significant excess of vCJD cases in young individuals. In addition, rates of blood donation and transfusion depend on age. Figure 1*a* shows the age-dependent donation rate for 1997 obtained from figures provided by the NBS (Det Norske Veritas 2003). Across all age groups, an average of 4300 units of blood were donated per 100 000 population per year, with 70% of donations received from those under 50 years of age. In contrast, transfusion-associated procedures are primarily undertaken in the elderly, with 66% undertaken in those aged 60 years and above (figure 1*b*; Wells *et al.* 2002). Finally, we must also allow for age-dependent excess mortality due to survival following surgical procedures incorporating transfusion, because infected patients may die before passing on the disease or developing clinical disease (figure 1*d*; Wallis *et al.* 2004).

## 3. A MATHEMATICAL MODEL

### 3.1. Basic model structure

The deterministic compartmental model for the transmission of vCJD infection in the UK has three components: (i) the primary epidemic through bovine-to-human transmission (i.e. through consumption of BSE-infected food material) which produces the initial background infection level for human-to-human transmission, (ii) the donation of blood which is packaged into blood units and stored for subsequent use in blood transfusions, and (iii) transmission through transfusions of infected blood into a susceptible host and their subsequent infection.

The first component described above determines bovine-to-human transmission in the initial primary epidemic and is modelled as an age- and time-dependent hazard of infection in which individuals are assumed to enter a carrier state (or not) with a fixed probability after infection. The parameters of this model are taken to equal updated maximum-likelihood estimates from a survival model for the primary epidemic (Clarke & Ghani 2005) fitted to data from clinical cases taken up until the end of

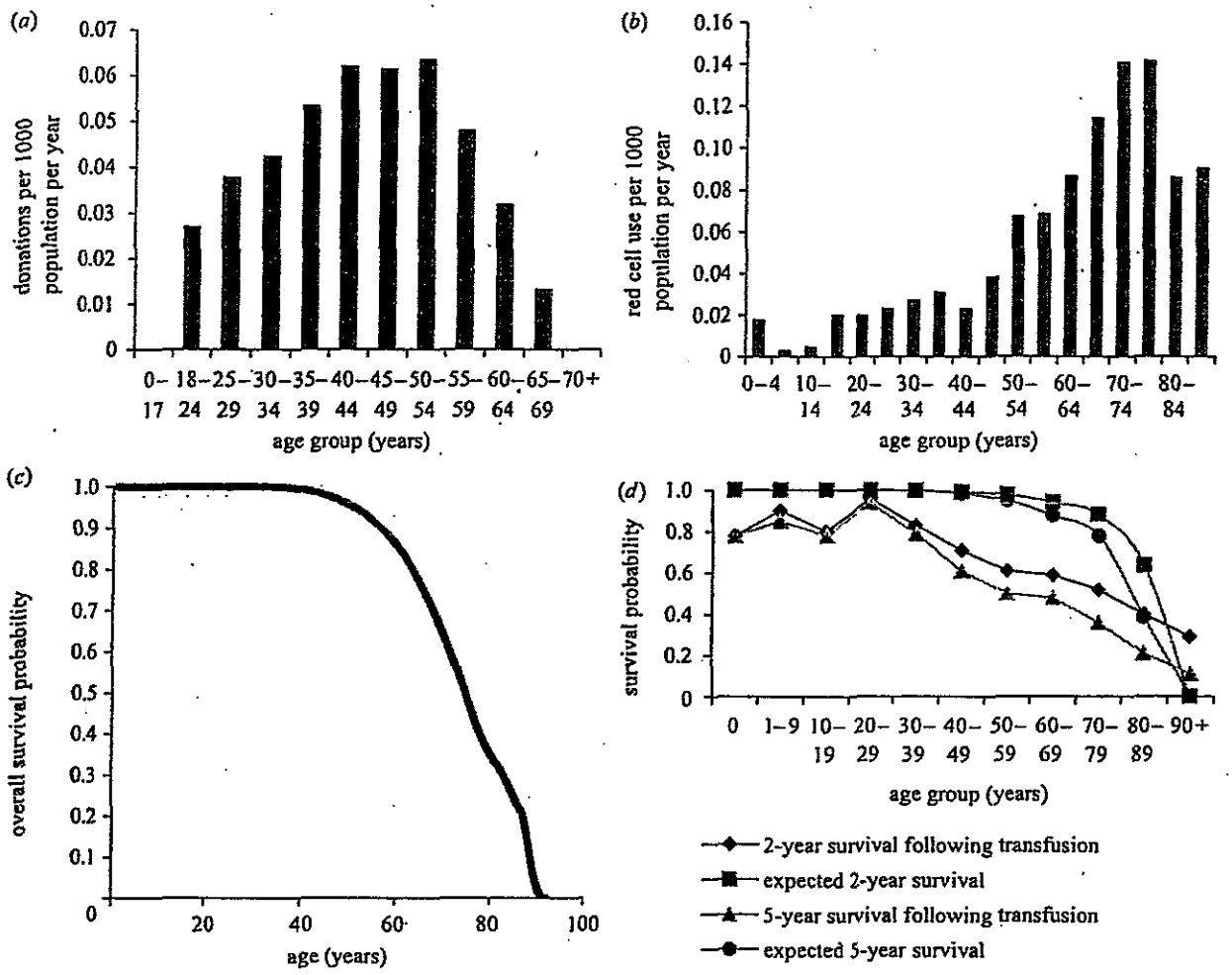


Figure 1. Data on blood supply and UK mortality rates. (*a*) Whole blood donation rates by age obtained from national figures provided by the NBS in 1997. (*b*) Red cell transfusion rates by age group obtained from a 28-day prospective observational study in the North of England (Wells *et al.* 2002). The destination of 9848 units (97% of expected blood use) was recorded. (*c*) The overall survival probability for the UK obtained from 1993 census data (Ghani *et al.* 1998). (*d*) 2- and 5-year survival probabilities by age group for patients receiving red cell transfusions from the prospective observational study in the North of England (Wallis *et al.* 2004). Corresponding estimates of the 2- and 5-year post surgery survival probabilities used in the mathematical model are also shown.

2005. Components (ii) and (iii) are two interrelated models describing blood-borne transmission of vCJD. UK blood stocks are treated as a pool of blood from which a fixed number of units are taken for procedures requiring blood transfusions; the rate at which blood is used and donated, estimated from the NBS data, ensures realistic storage times for donated blood units. For the secondary epidemic, we follow recent research and assume all genotypes are susceptible to infected blood (Bishop *et al.* 2006). The same research further suggests that infected blood may induce subclinical infection with an unknown carrier state probability, and so we also allow for this in the model.

### *3.2. Model specification*

The model is specified in continuous epidemic time indexed by $t$, with the assumption that $t=0$ corresponds to the origin of the BSE epidemic on 1 January 1980. Birth cohorts are defined according to the calendar year in which individuals were born and are indexed by $c$. We make the weak assumption (i.e. one having little effect on the results) that all individuals in a birth cohort were born at the start of that calendar year. Denote the time at which people in birth cohort $c$ were born by $t_c$, which take integer values corresponding to calendar years. For example, $t_{1980}=0$ for those born in 1980 and $t_{1970}=-10$ for those born in 1970. The age of those in birth cohort $c$ at time $t>0$ can thus be defined $a_{ct}=\lfloor t-t_c \rfloor$, where $\lfloor z \rfloor$ is the largest integer less than or equal to $z$.

Now let $X_c(t)$ denote the number of susceptible individuals in birth cohort $c$ at epidemic time $t$. For cohorts in which individuals were born prior to 1980, $X_c(0)$ is equal to the cohort size at the start of 1980. For cohorts in which individuals were born after 1980, $X_c(t_c)$ is equal to the number born during that year. Further, let $Y_{c,f}(t)$ and $Z_{c,f}(t)$ represent the number of primary- and blood-route-infected individuals in birth cohort $c$, respectively, where $Y_{c,f}(0)=Z_{c,f}(0)=0$ for all $c$ and $f$.

Subscript $f$ denotes infection status, where $f=0$ denotes subclinical infection and those with preclinical infection move successively through $F$ stages of incubation indexed by $f=1, \ldots, F$, with death occurring

immediately following stage $F$ (i.e. we ignore the lag between clinical diagnosis and death). Note that the incubation period is divided into multiple stages to ensure that they follow a Gamma distribution under the model; the stages do not have any intrinsic biological interpretation. Finally for the human population, the cumulative numbers of deaths at time $t$ from the primary and blood infection routes are denoted by $D_1(t)$ and $D_2(t)$, respectively.

The rate at which the size of each compartment changes in birth cohort $c$ at time $t$ is governed by the following set of differential equations:

$$X_c'(t) = -X_c(t)(\hat{\eta}_c(t)+\lambda_c(t)+\delta_c(t))$$

$$Y_{c,f}'(t) = \begin{cases} X_c(t)\hat{\omega}_1\hat{\eta}_c(t) - Y_{c,0}(t)\delta_c(t) & \text{if } f=0, \\ X_c(t)(1-\hat{\omega}_1)\hat{\eta}_c(t) - Y_{c,1}(t)(\delta_c(t)+\hat{\gamma}) & \text{if } f=1, \\ Y_{c,(f-1)}(t)\hat{\gamma} - Y_{c,f}(t)(\delta_c(t)+\hat{\gamma}) & \text{if } f>1, \end{cases}$$

$$Z_{c,f}'(t) = \begin{cases} X_c(t)\omega_2\lambda_c(t) - Z_{c,0}(t)\delta_c^*(t) & \text{if } f=0, \\ X_c(t)(1-\omega_2)\lambda_c(t) - Z_{c,1}(t)(\delta_c^*(t)+\xi) & \text{if } f=1, \\ Z_{c,(f-1)}(t)\xi - Z_{c,f}(t)(\delta_c^*(t)+\xi) & \text{if } f>1, \end{cases} \quad (3.1)$$

where $X_c'(t), Y_{c,f}'(t), Z_{c,f}'(t)$ are the derivatives of $X_c(t)$, $Y_{c,f}(t)$ and $Z_{c,f}(t)$, respectively, with respect to $t$; $\hat{\eta}_c(t)$ is the best estimate of the rate of primary infection; $\lambda_c(t)$ is the rate at which transfusion-acquired infections occur; $\delta_c(t)$ is the death rate from non-vCJD-related causes; $\delta_c^*(t)$ is the death rate among the transfusion recipient population; $\hat{\gamma}$ is the best estimate of the rate at which individuals leave primary incubation stage $f>0$; $\hat{\omega}_1$ is the best estimate of the proportion of primary infections that are subclinical; $\xi$ is the rate at which individuals leave transfusion-acquired incubation stage $f>0$; and $\omega_2$ is the proportion of subclinical transfusion-acquired infections.

Cumulative deaths from the primary and secondary infection routes increase at rates

$$D_1'(t) = Y_{+,F}(t)\hat{\gamma} \text{ and } D_2'(t) = Z_{+,F}(t)\xi, \quad (3.2)$$

where $D_1'(t), D_2'(t)$ denote the derivatives with respect to $t$ and a '+' subscript denotes summation over all levels of the obscured index.

For the blood population, let $U(t)$ and $C(t)$ denote the number of uncontaminated and contaminated blood units at $t$, where $V(t)=U(t)+C(t)$ is the blood population size and $U(0)=V(0)$, $C(0)=0$. We then make the further weak assumption that the population size is fixed over time, $V(t)=V$. Using this notation, the force of infection via the blood transfusion route above is given by the mean-field equation

$$\lambda_c(t) = W_c(t)\tau(t)\psi(a_{ct})\left(1-(1-\beta_2 C(t)/V)^{b(a_{ct})}\right), \quad (3.3)$$

where $a_{ct}$ is the age of those in birth cohort $c$ at time $t$ (defined above); $\tau(t)$ is the rate of procedures requiring transfusions; $\psi(a_{ct})$ is the proportion of these procedures carried out on those age $a_{ct}$; $\beta_2$ is the transmission probability for blood-route infection; and $b(a_{ct})$ is the average number of blood units user per transfusion on patients age $a_{ct}$. Finally, the weight $W_c(t)=N(t)/N_c(t)$ converts a per-age group rate into a per-birth cohort rate (required because each age group generally includes multiple birth cohorts).

Using a similar notation, the corresponding model for infection of the blood population at time $t$ is given by

$$\begin{aligned} U'(t) &= \sum_c X_c(t)\rho(a_{ct}) \\ &\quad - \sum_c N_c(t)\phi(t,a_{ct})U(t)/V, \\ C'(t) &= \sum_c (N_c(t)-X_c(t))\rho(a_{ct}) \\ &\quad - \sum_c N_c(t)\phi(t,a_{ct})C(t)/V, \end{aligned} \quad (3.4)$$

where $N_c(t)=X_c(t)+Y_{c,+}(t)+Z_{c,+}(t)$ is the size of the surviving population in birth cohort $c$ at time $t$; $\rho(a_{ct})$ is the rate at which people age $a$ donate blood units (blood units per person per year); and $\phi(t, a_{ct})$ is the rate at which blood units are used in red cell transfusions among those age $a_{ct}$ at time $t$. This last quantity is given by

$$\phi(t, a_{ct}) = W_c(t)\tau(t)\psi(a_{ct})b(a_{ct}). \quad (3.5)$$

### 3.3. Parameter values

Values for $\tau(t)$, $\psi(a)$, $b(a)$ and $\rho(a)$ were obtained from the data sources described in §2. The estimates of $\hat{\eta}_c(t)$ and $\hat{\omega}_1=0.1$ were obtained by fitting a previously developed survival model (Ghani *et al.* 2000; Clarke & Ghani 2005) to (i) the time- and age-stratified counts of vCJD deaths updated at the end of 2005 and excluding the two clinical cases assumed to be blood infected and (ii) the results from the survey of 12 674 tonsil and appendix tissues (Hilton *et al.* 2004), assuming that the diagnostic tests are sensitive only in the final 50% of the incubation period.

The death rate in the general population due to causes other than vCJD is calculated using estimates of population survivorship made by the Government Actuary's Department. Denote the survivorship until calendar year $u$ for those age $a$ by $S(u, a)$. Ignoring temporal variation in the population survivorship by setting $S(u, a)=S(a)$ and assuming a constant death rate within each age group, standard results give

$$\delta_c(t) = \begin{cases} -\log S(a_{ct}|a_{ct}-1) & \text{if } a_{ct}=1,\ldots,91, \\ -\log S(0) & \text{if } a_{ct}=0, \end{cases}$$

where $S(a|a-1)=S(a)/S(a-1)$ is the conditional survivorship function for interval $(a-1, a]$. Owing to low survivorship beyond age 91 years, we assume that everyone dies instantaneously on their 92nd birthday. The death rate among the transfusion recipient population is set to be $\delta_c^*(t)=e^{\xi}\delta_c(t)$, where $e^{\xi}=\exp(\xi)$ is the relative hazard such that $S^*(a+1)=S(a+1)^{\exp(\xi)}$. The value $\zeta=1.25$ was chosen to approximate Kaplan–Meier estimates of age-stratified survivorship (Wallis *et al.* 2004).

### 3.4. Self-sustaining epidemics and the basic reproduction number

In addition to the total size of the epidemic (as measured by the total number of people infected), it is of interest to establish whether the epidemic could be self-sustaining. The basic reproduction number ($R_0$) is

used to quantify this idea. It is defined as the expected number of infections caused by introducing one vCJD-infected individual into an otherwise susceptible population at the start of the epidemic. It has the property, in an infinite population, $R_0>1$ indicates that a self-sustaining epidemic is certain. More practically, in very large but finite closed populations, it indicates that a self-sustaining epidemic occurs with probability close to 1.

If we assume the ultimate size of the epidemic can only be small compared with the total population size, an analytical expression for $R_0$ can be derived using the method proposed by Dickmann *et al.* (1990). For the model described above, it can be shown that

$$R_0 = \sqrt{\beta_2 \sum_a \frac{N_a}{N} \sum_{u=0}^{91-a} \rho(a+u) S(a+u|a) \left\{ (1-\omega_2) e^{-u\xi} \sum_{r=0}^{F-1} \frac{\xi^r}{r!} k_r(u,a) + \omega_2 m(u,a) \right\}}, \tag{3.6}$$

where

$$k_r(u,a) = \begin{cases} \dfrac{(u+1)^r e^{-\xi} S(a+u+1|a+u) - u^r - r k_{r-1}(u,a)}{-\xi + \log S(a+u+1|a+u)} & \text{if } r = 0, \ldots, F-1, \\ 0 & \text{if } r = -1, \end{cases}$$

$$m(u,a) = \begin{cases} \dfrac{S(a+u+1|a+u)-1}{\log S(a+u+1|a+u)} & \text{if } S(u+a+1|a+u) < 1, \\ 1 & \text{otherwise,} \end{cases}$$

and $N_a/N$ is the proportion aged $a$ in 1980. The derivation of this expression is similar to that set out for a closely related model by Garske *et al.* (2006).

### 3.5. Interventions

In 1997/1998, the UK Department of Health responded to the theoretical risk of vCJD being spread by blood transfusion by requiring that all donated blood underwent leucodepletion to remove potentially infectious leucocytes. Subsequently, early in 2004 following discovery of the first two suspected blood-related vCJD infections, the Department of Health additionally excluded all those who were known to have had blood transfusions since 1980 from donating blood.

We investigate the potential effect of these two interventions by extending the mathematical model above to simulate their impact (the implementation is described in appendix A). Leucodepletion has been found to reduce infectious dose by 40% (Gregori *et al.* 2004). However, the effect this has on transmission coefficient $\beta_2$ depends on the relationship between dose and transmission, and on the threshold below which transmission cannot occur, both of which are unknown. Thus, we consider the two extremes: an 'optimistic' scenario in which leucodepletion is 60% effective, and a 'pessimistic' scenario in which it is 0% effective. For the donation ban, we consider an optimistic scenario of a 90% effective ban and a pessimistic 0% effective ban.

### 3.6. Sensitivity analysis

As described above, the parameters determining the UK population demographics and its blood supply were fixed at the estimates obtained using the data on survival, blood donation and transfusion. Epidemic scenarios are generated by varying the three remaining parameters: the transmission probability of disease, given the contact with infected blood (range 0–1); the proportion of transfusion-acquired infections entering a carrier state (range 0–1); and the ratio between the mean incubation periods of preclinical transfusion- and bovine-to-human-acquired vCJD (range 0–5). Note that the latter parameter corresponds to an absolute measure of length because the mean primary incubation period distribution is fixed.

Scenarios are generated at random using Latin Hypercube Sampling, i.e. drawing triples at random from the domain of the three parameters (Stein 1987). A batch of 10 000 scenarios was analysed to explore the scale and dynamics of the secondary epidemic. Scenarios were included only if they were consistent at the 95% level with the two clinical cases observed by the end of 2005. For simplicity, the scenarios were not constrained to match the third non-case found in 2004 because neither its infection time nor whether it is a pre- or subclinical infection is known. Latin hypercube sampling cannot be exhaustive, but further batches of 10 000 were analysed (results not shown) to ensure the original batch was representative of the parameter space.

## 4. RESULTS

### 4.1. Potential for self-sustaining epidemics

The results presented are taken from a single representative batch of 10 000 scenarios. In total, 7702 scenarios were accepted as consistent with the two clinical cases observed. In the scenario analysis, we first consider the potential size of the epidemic in the hypothetical situation that the Department of Health did not introduce leucodepletion or ban donations from former donors. The basic reproductive number ($R_0$) ranged between less than 0.001 and 1.35. The wide range incorporates both epidemic extinction and self-sustaining epidemics, and the range itself quantifies

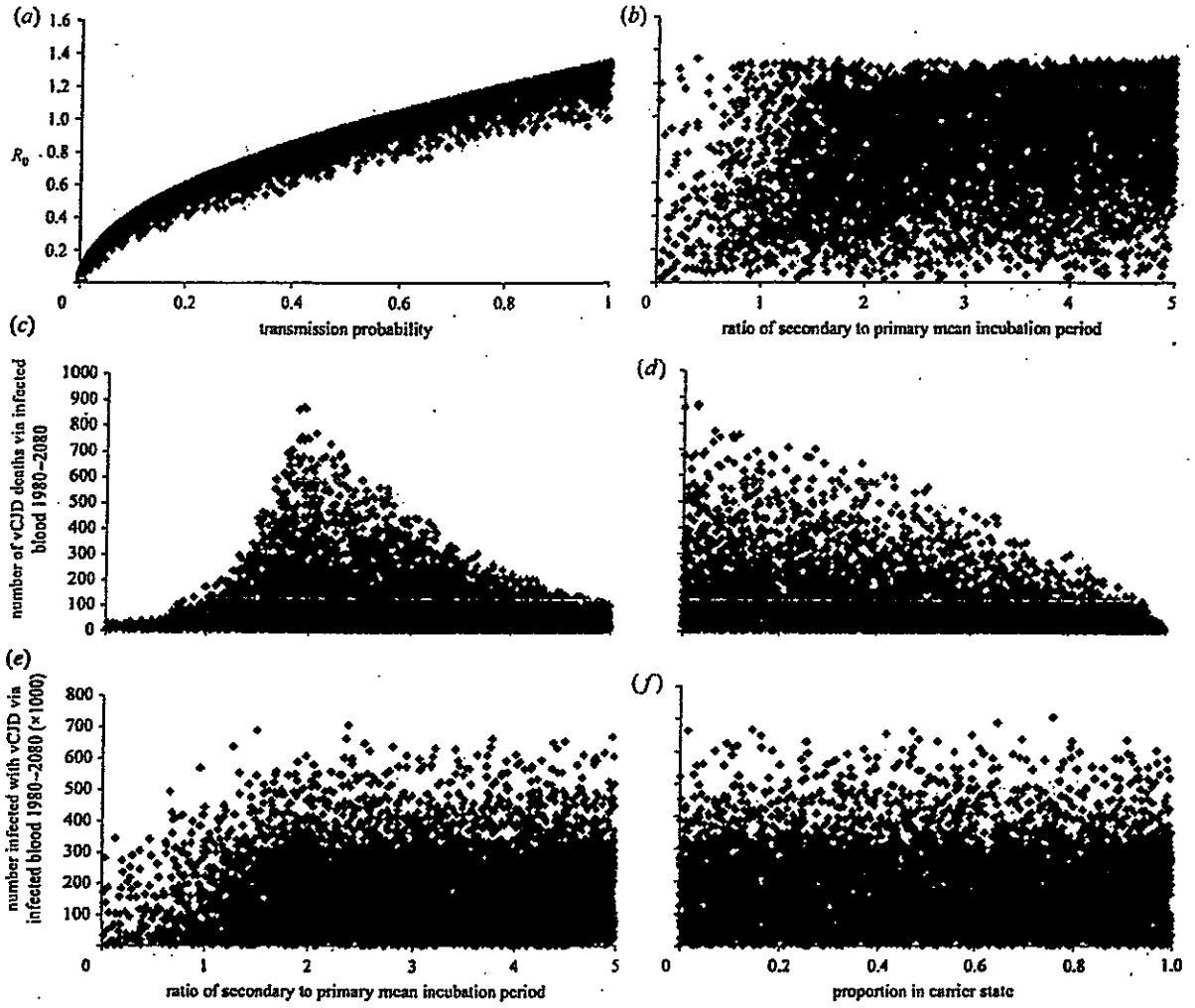


Figure 2. Sensitivity analysis. The relationship between $R_0$, epidemiological parameters and future infections and clinical cases without interventions. (*a*) $R_0$ and the transmission probability from blood. (*b*) $R_0$ and the ratio of the mean incubation period in those infected via blood transfusion to the mean incubation period in those infected via consumption of BSE-infected beef. (*c*) The total number of clinical cases by 2080 and the ratio of the mean incubation periods for those infected via blood transfusion and those infected via consumption of BSE-infected beef clinical cases by 2080. (*d*) The total number of clinical cases by 2080 and the probability of entering a carrier state after infection via blood transfusion. (*e*) The total number of people infected via blood transfusion by 2080 and the mean incubation period ratio. (*f*) The total number of infections by 2080 and the carrier state probability for blood-borne infections.

uncertainty about the transmissibility of vCJD via infected blood. If there was complete uncertainty, then we would have expected the upper bound for $R_0$ to be much higher. In the event, the worse-case scenario is limited by the rates and age distributions of those donating blood and receiving transfusions. Self-sustaining epidemics with $R_0 > 1$ occur when the transmission probability is high (clearly shown in figure 2*a*) and when the mean incubation period for transfusion-acquired (human-to-human) infection is longer than for primary (bovine-to-human) infections: the scatter of $R_0 > 1$ scenarios is sparse in the region of figure 2*b* where the ratio is less than 1. The latter scenario is biologically plausible because within-species transmission usually results in shorter incubation periods than between-species transmission (Kimberlin & Walker 1978; Bruce *et al.* 1994; Bruce 1996). Hence, it is unlikely that this route of transmission will give rise to a self-sustaining epidemic.

### *4.2. Potential scale of the epidemic*

Continuing with the hypothetical 'no intervention' situation, we now consider the size of the blood-borne vCJD epidemic up until 2080. The first row of table 1 contains the range of transfusion-acquired clinical case numbers. The worst-case scenario without any intervention results in an expectation of 871 deaths by 2080. The first factor limiting the epidemic is the constraint that scenarios should be consistent with the two observed cases infected by blood transfusions by 2006, which automatically excludes the most extreme scenarios.

We can explore the dynamics of the epidemic further to establish the remaining factors limiting epidemic potential. The relationship between the mean secondary incubation period and the total number of vCJD cases/deaths by 2080 is shown in figure 2*c*. Short secondary incubation periods correspond to small

Table 1. Upper bounds from sensitivity analysis. Range from scenario analysis of the expected number of deaths from vCJD contracted via blood transfusion. The intervention scenarios incorporate the ban on blood donations from those who have previously received blood and the introduction of leucodepletion in 1997 (effective leucodepletion reduces the transmission probability by 40%, while ineffective leucodepletion has no effect on the transmission probability). The ban on blood donations from those who have previously received a blood transfusion from 2004 onwards is assumed to be 90% effective). '0' indicates a small positive value less than 0.001.

| | clinical cases | | | |
|---|---|---|---|---|
| | 2005-2010 | 2005–2015 | 2005-2020 | 1980 2080 |
| no intervention | 0-19 | 0-52 | 0-104 | 0-871 |
| effective leucodepletion only | 0-17 | 0-40 | 0-67 | 0 294 |
| ban only | 0-19 | 0-45 | 0-100 | 0-569 |
| both leucodepletion and ban | 0-17 | 0–40 | 0—67 | 0-257 |

epidemics as one might expect. However, increasing secondary mean incubation periods correspond to decreasing epidemic sizes because age and non-vCJD post-transfusion mortality limits the number of future donations. Another factor limiting the epidemic size is the carrier state probability. Figure 2*d* shows the relationship between the probability of entering a carrier state and the total vCJD deaths. If the probability of entering a carrier state is close to one, the size of the epidemic is limited because most of those infected enter a carrier state and thus cannot die of vCJD. In contrast, if this probability is small then the majority of infections develop into clinical cases, with worst-case scenarios resulting if additionally the mean secondary incubation period is roughly twice that for primary infections.

If the epidemic size is expressed in terms of numbers infected rather than clinical cases then a slightly different picture emerges. From figure 2*e*, it can be seen that longer secondary incubation periods do not correspond to small epidemics, but that the worst case epidemic for longer mean incubation periods plateaus. In figure 2*f*, the distribution of epidemic sizes is independent of the value assumed for the probability of entering a carrier state, and so there is a weaker relationship between the number of people infected and clinical case numbers for scenarios in which the probability of entering a carrier state is close to 1. The level of the plateau comes partly from the rates and age distribution of blood use in the UK.

Now consider the effect of interventions. If leucodepletion was the sole intervention and reduced the transmission coefficient by 40%, from table 1 it can be seen that the worst-case scenario is reduced by 66% to 294 deaths. The subsequent introduction of the ban on donations from transfusion recipients causes a more modest reduction in the worst-case scenario from 294 to 257 deaths (assuming the ban is 90% effective). If the ban was introduced without leucodepletion then the worst-case scenario would lead to 569 deaths, a reduction of 35% from that without interventions.

For more biologically plausible scenarios in which the mean incubation period for transfusion-acquired cases is similar or shorter than that for primary cases, the maximum number of future cases is substantially smaller (46 compared to 171). Similarly, in scenarios where a large proportion of those infected enter a carrier state (>85%) the upper bound is 53 compared with 202 without interventions. The basic reproduction number has the same upper bound ($R_0$=1.35) in all scenarios and biologically plausible scenarios because high basic reproductive numbers tend to occur in scenarios in which there is a high probability of entering a carrier state. In all scenarios, a large proportion (30–60%) of clinical cases are expected to occur in those aged over 60 years (not shown), and the current prevalence of transfusion-acquired infection is expected to be low (16–27 infections per million population).

It is useful to know whether the pattern of the epidemic over the coming years can provide any clues as to the extent of transfusion-acquired infection. Figure 3 shows the evolution of the primary- and transfusion-acquired epidemics for two simulated scenarios in the absence of any interventions. In the first (figure 3*a*), $R_0$ is very low with few transfusion-acquired clinical cases over time and a continued decline in primary cases from 2005 to 2010. In the second scenario (figure 3*b*), $R_0>1$ and the clinical case distribution peaks in 20 years time, with a long tail stretching over decades. These results suggest that it would be difficult to identify the degree of transfusion-acquired infection on the basis of the cases alone in the coming years. Caution is also required in drawing firm conclusions from a second peak, given the other remaining uncertainties in the epidemiology of vCJD, in particular, the possibility of wider genetic susceptibility.

## 5. DISCUSSION

We have produced upper bounds for the size of the vCJD epidemic via blood transfusion, given current understanding of the disease and making many pessimistic assumptions about the disease epidemiology. An upper bound of the order of hundreds rather than millions of deaths clearly demonstrates that the data we have are informative about the scale of the epidemic. Our results demonstrate that there is unlikely to be a self-sustaining secondary epidemic of vCJD due to blood transfusions. This is particularly apparent when considering only biologically plausible scenarios. We also found that the early introduction of leucodepletion and the subsequent ban on donors donating were timely and effective interventions, leading to substantial reductions in the upper bound for the ultimate size of the secondary epidemic.

The major limiting factor for the secondary epidemic appears to be the rate at which blood is donated and used in transfusions, which is low enough to limit the potential for onward transmission. In addition, it appears that the age of donors (below 60 years) and the age of transfusion recipients (above 60 years) also limits the potential for a self-sustaining epidemic: after one generation of infections (from young to old), the

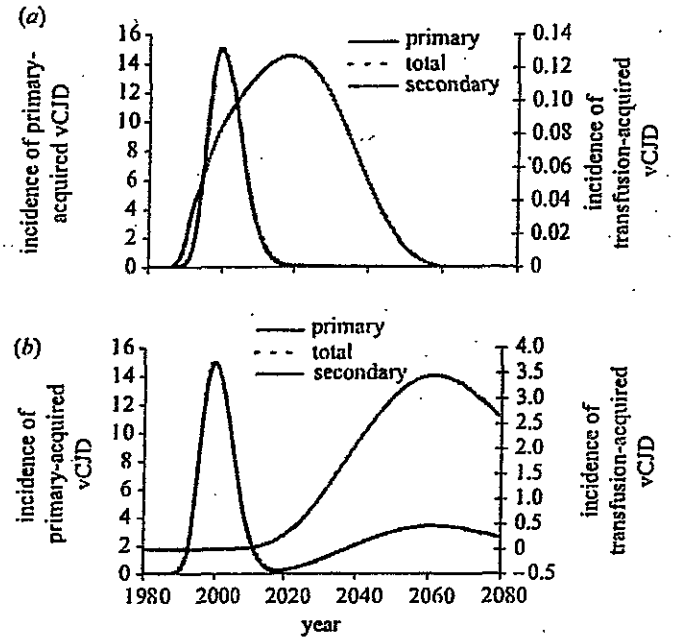


Figure 3. A sample epidemic. Two scenario epidemics. (*a*) An epidemic with low $R_0=0.03$ showing that the vast majority of vCJD clinical cases arise via primary infection. (*b*) An epidemic with high $R_0=1.3$ showing a multi-modal distribution of vCJD cases with the first peak occurring in 2000 from primary transmission and a second, much lower, peak in 2060 from transfusion-acquired infection. In both the scenarios, it is assumed that 10% of those infected via blood transfusion go on to develop clinical disease and that no interventions have taken place.

chance of onward infection (from old to young) is reduced. The basic reproductive number for transmission via this route is further reduced owing to the reduction in life expectancy of those infected via blood transfusions owing to competing morbidities.

It is important to acknowledge that our results must be interpreted carefully. The problem of predicting the secondary vCJD epidemic is one in which uncertainty is attached to most of the key parameters. Thus, it is not possible to provide fully robust predictions with the usual statistical rigour. What we do provide, under plausible assumptions regarding the transmission route, genotype, incubation period and carrier state for vCJD, together with assumptions about the effectiveness of current interventions, is a bound for the order of magnitude of the epidemic in the worst case.

The advantage of our sensitivity analysis approach is that all the sources of uncertainty are clearly set out and their combined effect on predictions quantified. However, there are sources of uncertainty we have not considered. While there are additional risks from blood components other than red blood cells (e.g. the past use of UK-derived pooled blood products such as the clotting factors received by haemophiliacs; Dolan 2006), the vast majority of blood use is for red cell transfusion. The important influence of absolute rates of blood use/donation in secondary epidemic dynamics would suggest that such additional risks could only be expected to modestly increase our upper bounds if included in our analysis. Additionally, we have not considered here that a secondary epidemic could arise via other routes of human-to-human transmission, in particular through contaminated surgical instruments. This risk has been considered elsewhere (Garske *et al.* 2006), but to fully assess the risk from human-to-human routes, further work will be required to combine these models because the overlap between human-to-human routes is substantial (approx. 40% of blood transfusions occur during surgery; Wells *et al.* 2002).

The ultimate aim of modelling work should be to provide robust estimates of the secondary vCJD epidemic via human-to-human transmission. The model results presented here demonstrate that one of the major uncertainties limiting assessment of the potential scale of this epidemic is the lack of data on the prevalence of preclinical and subclinical vCJD infection in the population. A large study is currently underway to better assess the prevalence of asymptomatic infection via detection of abnormal prion protein in tonsil tissues removed during routine operations. Recent findings may eventually shed further light on the relationship between genotype, susceptibility, incubation period and the carrier state (Ironside *et al.* 2006). As more cases emerge, further work will be undertaken to estimate the parameters of the mathematical model from case data and new data on infection prevalence. Using Bayesian statistical techniques, uncertainties such as those about the infection route for each case can be incorporated, and these robust estimates will be important in refining the sensitivity analysis presented here to predict the future scale of this major public health concern.

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Health. The views expressed in this publication are those of the authors and not necessarily those of the Department of Health.

## APPENDIX A

### *A.1. Leucodepletion*

As described in §3.3, the optimistic scenario is taken to be a 60% effective ban, corresponding to a 40% reduction in the transmission coefficient. Let $t^{\mathrm{L}}=16$ be the time at which leucodepletion was first introduced, and substitute all occurrences of $\beta_2$ in the model above by $\beta_2(t)=(0.6)^{i_{\mathrm{L}}}(t)\beta_2$, where $i_{\mathrm{L}}(t)=1$ if $t\geq t^{\mathrm{L}}$ or 0 otherwise.

### *A.2. Ban on blood donations*

The ban excludes those who received blood at any time since 1980 from donating blood after 2004, i.e. $t\geq 24$. We assume (optimistically) this ban is 90% effective. We do not explicitly exclude those who receive blood but are not themselves infected because we assume any shortfall are offset by eligible donors from the susceptible population; nor do we explicitly exclude those transfusion recipients who were infected by the primary route because the number of such people is small. Let $t^{\mathrm{B}}=24$ be the time at which the ban on blood donations from transfusion recipients was introduced, then equation (3.4) can be rewritten as

$$U'(t)=\sum_c X_c(t)\rho(a_{ct})P(a_{ct})^{i_{\mathrm{B}}(t)} - \sum_c N_c(t)\phi(t,a_{ct})U(t)/V,$$

$$C'(t)=\sum_{c,f}\left(Y_{c,f}(t)P(a_{ct})^{i_{\mathrm{B}}(t)}+(1-i_{\mathrm{B}}(t)Z_{c,f}(t)\right)\rho(a_{ct}) - \sum_c N_c(t)\phi(t,a_{ct})C(t)/V,$$

where $P(a_{ct})$ is the proportion of those age $a_{ct}$, who have not received any blood since 1980, and $i_{\mathrm{B}}(t)=0.9$ if $t\geq t^{\mathrm{B}}$ or 0 otherwise. In practice, we assume that $P(a)\approx 1$ and so $Y_{c,f}(t)P(a_{ct})\approx Y_{c,f}(t)$ and $X_c(t)P(a_{ct})\approx X_c(t)$. The exclusion from March 2004 onwards of those who have received blood transfusions after 1980 from donating blood will mean the effective reproduction number (i.e. the basic reproduction number immediately following intervention) is close to 0.

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| 一般的名称 | | | 研究報告の公表状況 | Transmission of Elk and Deer Prions to Transgenic Mice.<br>Tamguney, G. et al.,<br>J. Virol., 80; 9104-9114 (2006) | 公表国<br>米国 | |
| 販売名(企業名) | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 本論文はエルクとシカの脳から単離した慢性消耗病(CWD)プリオンのトランスジェニック(Tg)マウスへの感染成功例を示している。この目的のために，シカまたはエルクのプリオンタンパク質(ElkPrP，DePrpPp)のどちらかを発現する Tg マウスが作成された。これらの Tg マウスでは，マウス PrP は発現されず，シカ科の PrP のみ発現していた。いずれの系統の Tg マウスも自然発生の神経疾患は発症しなかった。シカ脳組織の調製液から，疾病に関与する PrP のレベルがシカ間で大きく異なることが示唆された。エルクとシカの CWD を接種した Tg マウス(ElkPrP または DePrP)では感染率が 100%であった(接種した全マウスで発症)。プリオン病の診断は，免疫組織化学と組み合わせた組織学的分析で行い，罹患したマウスの脳内に多数のプリオン・アミロイド斑が示された。Tg マウス(ElkPrP)では 180-200 日の間に，Tg マウス(DePrP)では 300-400 日の間にプリオン病が発症した。平均潜伏期間は神経病理学的損傷の変化をもたない次世代マウスでは短くなったが，導入 PrP の発現レベルとは反比例した。野生型マウス PrP を過剰発現した Tg マウス(MoPrP)にシカの脳組織を接種したところ，8 匹中 1 匹だけが感染した。この症例では，神経解剖学上の脳病変の特徴は，Tg マウス(ElkPrP 及び DePrP)で観察されるものとは異なっていた。対照的に，ウシ，ヒツジ，またはヒト PrP を過剰発現した Tg マウスへ CWD プリオンを接種したところ，500 日以上経ってもいかなる症状も発現しなかった。これらの結果から，エルクとシカの CWD プリオンはシカ科間で容易に感染することが確認された。 | BYL-2007-0247 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本文献ではエルクとシカの CWD プリオンはシカ科間で容易に感染することが確認された。CWD のウシまたはヒトへの感染を示す証拠は見つかっておらず，現時点では感染リスクは極めて低いと考える。 | これまでに，種の壁を越えたという報告例はないため，現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。 |

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# Transmission of Elk and Deer Prions to Transgenic Mice†

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**Chronic wasting disease (CWD) is a fatal prion disease in deer and elk. Unique among the prion diseases, it is transmitted among captive and free-ranging animals. To facilitate studies of the biology of CWD prions, we generated five lines of transgenic (Tg) mice expressing prion protein (PrP) from Rocky Mountain elk (*Cervus elaphus nelsoni*), denoted Tg(ElkPrP), and two lines of Tg mice expressing PrP common to white-tailed deer (*Odocoileus virginianus*) and mule deer (*Odocoileus hemionus*), denoted Tg(DePrP). None of the Tg(ElkPrP) or Tg(DePrP) mice exhibited spontaneous neurologic dysfunction at more than 600 days of age. Brain samples from CWD-positive elk, white-tailed deer, and mule deer produced disease in Tg(ElkPrP) mice between 180 and 200 days after inoculation and in Tg(DePrP) mice between 300 and 400 days. One of eight cervid brain inocula transmitted disease to Tg(MoPrP)4053 mice overexpressing wild-type mouse PrP-A in ~540 days. Neuropathologic analysis revealed abundant PrP amyloid plaques in the brains of ill mice. Brain homogenates from symptomatic Tg(ElkPrP) mice produced disease in 120 to 190 days in Tg(ElkPrP) mice. In contrast to the Tg(ElkPrP) and Tg(DePrP) mice, Tg mice overexpressing human, bovine, or ovine PrP did not develop prion disease after inoculation with CWD prions from among nine different isolates after >500 days. These findings suggest that CWD prions from elk, mule deer, and white-tailed deer can be readily transmitted among these three cervid species.**

Prions are transmissible pathogens that accumulate in the central nervous system (CNS) and cause fatal neurodegeneration (34). Prions are composed of an alternatively folded isoform of the prion protein (PrP), denoted $PrP^{Sc}$. The precursor of $PrP^{Sc}$ is a cellular protein designated $PrP^{C}$ that is encoded by a chromosomal gene. Prion diseases afflict humans as well as livestock, such as cattle, goats, and sheep; additionally, prions cause CNS disease in captive and wild populations of deer and elk (51, 53). In contrast to scrapie of sheep and goats, bovine spongiform encephalopathy (BSE) in cattle has been transmitted to humans and has killed more than 170 teenagers and young adults as variant Creutzfeldt-Jakob disease (vCJD) (44, 49, 51, 52). BSE prions have been experimentally transmitted to sheep and appear to be transmitted naturally among sheep (6). Recently, BSE prions were found in goats (14).

The transmission of BSE prions to humans has elevated concern about the possibility of the zoonotic transmission of chronic wasting disease (CWD) from deer and elk to humans (5, 56). Hunters and other consumers of venison are potentially at risk to acquire prion disease from infected deer and elk. CWD was first observed in 1967 in cervids and was recognized as a prion disease a decade later (54). CWD has been reported in 14 U.S. states and 2 Canadian provinces.

The epidemiology of CWD is unclear. In contrast to BSE and scrapie, CWD is highly transmissible among cervids. In some captive mule deer (*Odocoileus hemionus*) herds, 90% of the animals have been reported to be infected with CWD prions (28). The prevalence of CWD cases in free-ranging deer populations can be up to ~30% (53). The number of cases of CWD that arises spontaneously and then spreads horizontally, however, is unknown. Commercial farming and trade with cervids may foster horizontal transmission of CWD. It seems likely that, as surveillance improves, the known geographic distribution of CWD will increase. Cases of CWD have been reported in South Korea where elk had been imported from Canada (21). Furthermore, free-ranging elk and deer occasionally share the same pastures with cattle and sheep. It is therefore of concern whether CWD prions can be transmitted to livestock and on to humans. The passage of prions into a new host species can alter the host range: hamsters are resistant to CWD prions from deer and elk but are susceptible to CWD prions previously passaged in ferrets (4).

While our work was in progress, two reports appeared describing the transmission of CWD prions to transgenic (Tg) mice expressing cervid PrP (9, 23). The first study showed transmission of CWD prions to Tg(CerPrP) mice expressing the S2 PrP allele (GenBank accession no. AF009180) of mule deer, and the second study reported transmission of CWD prions to Tg12 mice expressing the eGMSE PrP allele (GenBank accession no. AF156183) of Rocky Mountain elk (*Cervus elaphus nelsoni*). The respective alleles are expressed exclusively in deer or elk and give rise to PrP molecules that differ only at residue 226, which is glutamine in deer PrP and glutamate in elk PrP. Transmission of a CWD brain sample from Rocky Mountain elk to Tg(CerPrP) mice resulted in incubation times of ~240 days. Transmission times of several CWD brain samples from mule deer were between 230 and 260 days. On second passage, the incubation time was 160 days in mice

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homozygous for the transgene (9). The second study showed transmission of CWD prions from elk to Tg12 mice within 120 to 140 days; no change in incubation time was observed on second passage (23).

In the findings reported here, we describe studies with Tg mice expressing either elk PrP [Tg(ElkPrP)] or deer PrP [Tg(DePrP)]. The Tg(ElkPrP) mice used in this study express the same PrP as Tg12 mice (23), and the Tg(DePrP) mice express the same PrP as Tg(CerPrP) mice (9). We generated four lines of Tg(ElkPrP) mice, one of which was bred to homozygosity, and two lines of Tg(DePrP) mice. We inoculated all lines with CWD prions from elk ($n = 1$). Additionally, we inoculated Tg(ElkPrP) and Tg(DePrP) lines with CWD prions from mule deer ($n = 2$) and white-tailed deer (*Odocoileus virginianus*; $n = 2$). Tg(ElkPrP) mice succumbed to prion disease within 180 to 200 days and Tg(DePrP) mice, with slightly lower DePrP transgene expression levels, within 300 to 400 days. Neuropathologic analysis showed spongiform degeneration, florid PrP amyloid plaques, and astrocytic gliosis in ill mice.

Based on the data reported here and those of others cited above, CWD prions from elk, mule deer, or white-tailed deer seem to be equally transmissible among these three cervid species. In addition, CWD prions do not readily transmit disease to Tg mice expressing human, bovine, or ovine PrP. Whether CWD prions cause disease in humans or livestock remains uncertain.

## MATERIALS AND METHODS

**Source of transgenic mice.** Unless otherwise specified, all Tg mice originated from Zrch.*Prnp*$^{0/0}$ mice, which do not express endogenous PrP from mouse (MoPrP) (10). Tg(BoPrP)4092 and Tg(OvPrP,VRQ)338 mice are homozygous for the respective transgene; all other lines used in this study are hemizygous. Tg(HuPrP)440, Tg(BoPrP)4092, Tg(BoPrP)4125, and Tg(MoPrP)4053 mice have been described previously (39, 42, 46, 47). Transgenic lines were maintained by breeding with FVB/*Prnp*$^{0/0}$ mice, except for Tg(MoPrP)4053, in which the endogenous *Prnp* gene was maintained by breeding with FVB mice (Charles River Laboratories, Wilmington, MA). Tg(OvPrP,VRQ)338 mice were a generous gift from H. Laude (26).

The PrP open reading frame (ORF) for ElkPrP was PCR amplified from elk tissue using the sequence-specific primer pair 5′-GTCTGTCGACGATG GTGAAAAGCCACATAGGC-3′ and 5′-GTCCTCGAGCTATCCTACTAT GAGAAAAATGAG-3′. DePrP was obtained by introducing a E226Q mutation into ElkPrP by site-directed mutagenesis using a QuikChange Multi Site-Directed mutagenesis kit (Stratagene, La Jolla, CA) and the mutagenesis primer 5′-CACCCAGTACCAGAGAGAATCCCAGGCTTATTACCAAAG AGGGG-3′. Complete sequences of ORF constructs were determined and archived by using Vector NTI Advance software (Invitrogen Corp., Carlsbad, CA). Tg(ElkPrP)12577, Tg(ElkPrP)12580, Tg(ElkPrP)3934, Tg(ElkPrP)12584, Tg(DePrP)10945, and Tg(DePrP)10969 mice were generated using the cosSHa.Tet cosmid vector for transgenic expression as described previously (41). Only one line, Tg(ElkPrP$^{+/+}$)3934, was made homozygous for the transgene by intercrossing Tg(ElkPrP)3934 mice. Expression levels of PrP$^{C}$ in the brains of Tg(ElkPrP) and Tg(DePrP) mice were determined by dot blotting using serial dilutions of homogenate and were compared to that of wild-type (WT) FVB mice (40). PrP was detected with the humanized recombinant fragment antibody (recFab) HuM-P (39) and developed with an enhanced chemiluminescent detection system (Amersham Biosciences, Piscataway, NJ) (44). The recFab HuM-P was expressed and fermented in *Escherichia coli* 33B6 competent cells and purified as described previously (33).

**Prion isolates and transmission studies.** The RML prion strain was derived from the Chandler isolate (12) passaged in CD-1 mice. The BSE isolate PG31/90 was obtained from John Wilesmith at the Central Veterinary Laboratory, Weybridge, United Kingdom. The sporadic CJD (sCJD) MM129 prion isolate HU00178 was obtained from a patient who was diagnosed with sCJD and whose PrP ORF revealed no mutations and methionine homozygosity at residue 129. The sheep scrapie isolate no. 027, derived from a Suffolk sheep with the ARQ genotype, was obtained from USDA. CWD elk isolates 03-12609 (Elk1), 03-01495 (Elk2), and 03-01483 (Elk3); mule deer (MD) isolates 03-12776 (MD1), 03-11714 (MD2), and 03-12812 (MD3); and white-tailed deer (WTD) isolates 03-12473 (WTD1), 8527 (WTD2), and 11993 (WTD3) were collected at the Wildlife Research Center in Fort Collins, Colorado. CWD elk isolate no. 99RA146 (Elk4) was obtained from Allen Jenny, formerly at the National Veterinary Services Laboratory, Ames, IA. Elizabeth Williams, formerly at the Wyoming State Veterinary Laboratory, University of Wyoming, Laramie, provided the following CWD isolates: nos. 99W4049 (Elk5), 99W2864 (Elk6), 99W4050 (Elk7), 98W770 (MD4), 98W1243 (MD5), 98W6679 (WTD4), 94W3471 (WTD5), and 95W9717 (WTD6).

Transmissions of prion isolates to Tg mice were performed as previously described (43). For samples Elk1, MD1, MD3, WTD1, and WTD2, 15% (wt/vol) brain homogenates were prepared in $Ca^{2+}$- and $Mg^{2+}$-free phosphate-buffered saline (PBS; pH 7.4) by three 75-s cycles in a reciprocal homogenizer (Mini-BeadBeater-8; BioSpec Products, Inc., Bartlesville, OK) as described previously (38, 39). The resulting homogenate was diluted to a final concentration of 1% (wt/vol) using PBS containing 5% (wt/vol) bovine albumin Fraction V (ICN) and 0.5 U/ml penicillin (Sigma, St. Louis, MO) and with 0.5 μg/ml streptomycin (Sigma). For all other inocula, 10% (wt/vol) brain homogenates in PBS were obtained by 10 repeated extrusions through syringe needles of successively smaller sizes, from 22 to 18 gauge. For inoculation, brain homogenates were further diluted in 5% (wt/vol) bovine albumin Fraction V and PBS to obtain a final 1% (wt/vol) brain homogenate. Mice were inoculated in the right parietal lobe with 30 μl of the 1% (wt/vol) brain homogenate using a 27-gauge, disposable hypodermic syringe. The clinical status of the mice was monitored daily, while the neurologic status was assessed three times per week. Animals were euthanized following evidence of progressive neurologic dysfunction (11, 40).

**Preparation of brain homogenates.** For Western blotting analysis, 10% (wt/vol) brain homogenates were prepared in PBS and 4% (wt/vol) Sarkosyl by three 75-s cycles in a reciprocal homogenizer (Mini-BeadBeater-8). The resulting homogenate was diluted to a final 5% (wt/vol) using PBS containing 4% (wt/vol) Sarkosyl. Homogenates were clarified by centrifugation at 500 × *g* for 5 min on a tabletop centrifuge. Samples of 5% brain homogenates were incubated with 20 μg/ml of proteinase K (PK) (Invitrogen) for 1 h at 37°C. The reaction was stopped with 2 mM phenylmethylsulfonyl fluoride (PMSF). Samples were centrifuged at 100,000 × *g* for 1 h at 4°C. Pellets were resuspended in 10 mM Tris-HCl (pH 8.0), 0.15 M NaCl, 0.5% (wt/vol) NP-40, and 0.5% (wt/vol) sodium deoxycholate. An equal volume of 2× sodium dodecyl sulfate sample buffer was added (25), and the mixture was boiled for 5 min prior to electrophoresis. Sodium dodecyl sulfate gel electrophoresis and Western blotting were performed as previously described (44). PrP was detected with the recFab HuM-P and developed with an enhanced chemiluminescent detection system (Amersham Biosciences). To determine the glycoform ratios of Western blot signals on exposed and developed film, we scanned the film using Kodak image station 440 CF (Kodak) and then quantified the signals using Kodak molecular imaging software (v. 4.0.3).

**Preparation of brain homogenates for the CDI.** For biochemical analysis only, slices from cervid brainstems weighing 250 to 350 mg were homogenized to a final concentration of 15% (wt/vol) in PBS containing 4% (wt/vol) Sarkosyl by three 75-s cycles in a reciprocal homogenizer Mini-BeadBeater-8. The resulting homogenate was diluted to a final 5% (wt/vol) using PBS containing 4% (wt/vol) Sarkosyl. The diluted samples were digested with 10 μg/ml PK for 1 h at 37°C using the shaker. After a clarification spin at 500 × *g* for 5 min at room temperature (RT) in a drum rotor (Jouan, Milford, MA), the samples were mixed with stock solution containing 10% phosphotungstate and 85 mM $MgCl_2$, pH 7.4, to obtain a final concentration of 0.31% sodium phosphotungstate and 2.6 mM of $MgCl_2$. After a 1-h incubation at 37°C on a rocking platform, the samples were centrifuged at 14,000 × *g* in a Jouan MR23i centrifuge for 30 min at RT. The resulting pellets were resuspended in $H_2O$ containing protease inhibitors (0.5 mM PMSF; aprotinin and leupeptin, 2 μg/ml each) and assayed by the conformation-dependent immunoassay (CDI).

**Sandwich CDI for cervid PrP$^{Sc}$.** The CDI data described in this paper were generated with recFab HuM-P labeled with Eu chelate of *N*-(*p*-isothiocyanatobenzyl)-diethylenetriamine-$N^1,N^2,N^3,N^3$-tetraacetic acid (DTTA) at pH 8.5 for 16 h at RT according to the manufacturer's protocols (Wallac, Inc., Turku, Finland). The final Eu/Fab molar ratio was 4.3.

The principle, development, calibration, and calculation of cervid PrP$^{Sc}$ concentration from CDI data have been described previously (38, 39). Briefly, each sample was divided into two aliquots: (i) untreated (designated native [N]) and (ii) mixed to a final concentration of 4 M guanidine hydrochloride and heated for 5 min at 80°C (designated denatured [D]). Both samples were immediately diluted 20-fold with $H_2O$ containing protease inhibitors (5 mM PMSF; aprotinin

and leupeptin, 4 μg/ml each), and aliquots were loaded on a 96-well, black polystyrene plate (Packard, Meriden, CT) that was coated overnight with 5 μg/ml of recFab HuM-D18 (33) in sodium phosphate buffer (pH 7.4) and blocked with Tris-buffered saline (pH 7.8) containing 0.25% (wt/vol) bovine serum albumin and 0.1% (wt/vol) Tween 20 for 1 h at RT. The plates containing native and denatured aliquots of each sample were then incubated for 2 h at RT. The plates were washed three times with Tris-buffered saline (pH 7.8) containing 0.05% (vol/vol) Tween 20, incubated with 0.25 μg/ml of Eu-labeled recFab HuM-P at RT for 2 h, and then developed after seven washing steps in the enhancement solution provided by the europium label supplier (Wallac, Inc.). The signal was counted on a Discovery dual-wavelength, time-resolved fluorometer (Packard). As a standard, we used denatured recombinant mouse-bovine PrP (MBo2M) (43). Each plate contained positive and negative controls prepared from pooled CWD-infected or uninfected brains. The results were expressed as (D − N), the difference of the time-resolved fluorescence (TRF) results for D and N aliquots, measured in counts per minute. The concentration of cervid $PrP^{Sc}$ is directly proportional to the (D − N) value (37–39, 48).

**Neuropathology.** For neuropathologic analysis, brains were removed rapidly from animals and either immersion fixed in 10% buffered formalin or frozen on dry ice. Paraffin-embedded brain sections of 8 μm were stained with hematoxylin and eosin for evaluation of neurodegeneration. Immunohistochemical detection of $PrP^{Sc}$ on formalin-fixed, paraffin-embedded tissue sections was performed by the hydrolytic autoclaving method and by using recFab HuM-P for PrP detection (30). Histoblotting was performed using 10-μm-thick frozen coronal sections that were blotted onto nitrocellulose membranes and processed for immunohistochemistry using recFab HuM-P directed against PrP (45). Reactive astrocytic gliosis was evaluated using peroxidase immunohistochemistry with a rabbit antiserum to glial fibrillary acidic protein (GFAP; Dako, Carpinteria, CA), as previously described (29).

## RESULTS

**Construction of Tg(ElkPrP) and Tg(DePrP) mice.** In order to obtain mice that are susceptible to CWD prions, we used the cosSHa.Tet vector to generate Tg mice that express ElkPrP or DePrP on the *Prnp*$^{0/0}$ background (41). Tg(ElkPrP) mice express ElkPrP with methionine at codon 132 (GenPept accession number AAF80282), which is commonly associated with CWD in elk (31). Tg(DePrP) mice express a PrP common to mule and white-tailed deer (GenPept accession numbers AAC33174 and AAF80284, respectively) (8, 18, 20, 32). Thus, both Tg(ElkPrP) and Tg(DePrP) express PrP with D20, Q95, G96, A116, M132, and S225 and that differs only at codon 226, which encodes glutamate in ElkPrP and glutamine in DePrP.

As shown in Table 1, we observed five lines of Tg mice overexpressing ElkPrP and two lines overexpressing DePrP for spontaneous disease. One line of uninoculated Tg(DePrP) mice was observed for ~650 days and the other for >600 days; neither line showed signs of disease. None of the uninoculated Tg(ElkPrP) mice under observation exhibited signs of disease; four lines of Tg(ElkPrP) mice were observed for >600 days and one additional line for >480 days. These healthy control Tg mice appear to demonstrate that mice tolerate the expression of cervid PrP without measurable illness.

TABLE 1. Uninoculated Tg mice expressing cervid PrPs

| Transgenic line | Cervid PrP expression (*n*-fold) | Age (days)[b] | No. of animals ill/no. of animals observed |
|---|---|---|---|
| Tg(ElkPrP)12577 | 2 | >618 | 0/8 |
| Tg(ElkPrP)12580 | 2 | >597 | 0/5 |
| Tg(ElkPrP)3934 | 1.5[a] | >603 | 0/6 |
| Tg(ElkPrP$^{+/+}$)3934 | 3 | >487 | 0/6 |
| Tg(ElkPrP)12584 | 3 | >606 | 0/5 |
| Tg(DePrP)10945 | 1 | >611 | 0/6 |
| Tg(DePrP)10969 | 1 | >642 | 0/8 |

[a] Half of the level of ElkPrP expression in Tg(ElkPrP$^{+/+}$)3934 mice.
[b] Age of the youngest animal when the experiment was terminated.

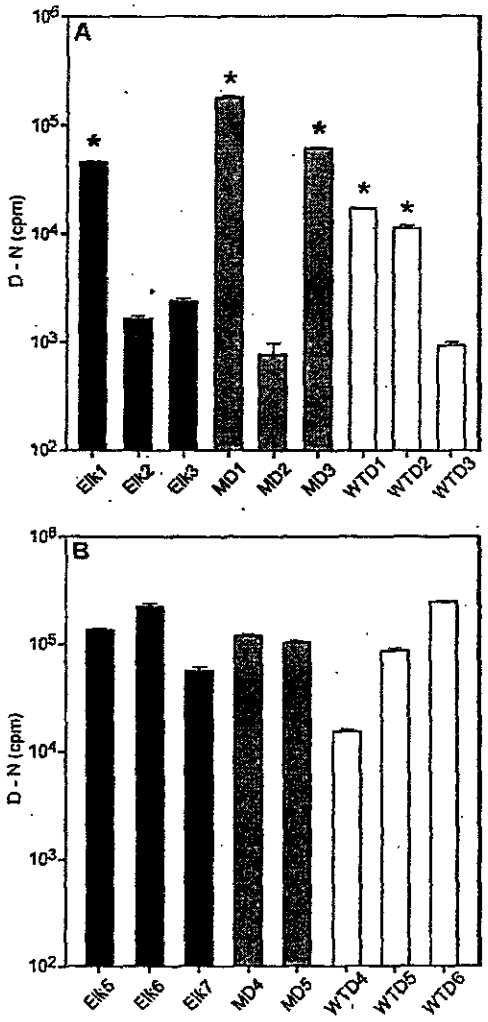


FIG. 1. Brainstem samples from 17 confirmed CWD cases in elk, mule deer, and white-tailed deer were assayed by CDI for relative $PrP^{Sc}$ titers. The (D − N) value is directly proportional to the amount of infectious prions within each sample and is depicted in logarithmic scale (38, 39). Bars represent the averages ± standard deviations (SD) obtained from three independent measurements. The cutoff (D − N) value of 314 cpm was calculated by [median + 5(SD)] and determined from 40 brainstem samples of normal deer and elk tested by the sandwich CDI in duplicate (39). Individual CWD isolates differed 10- to 100-fold in their $PrP^{Sc}$ content. (A) Only samples with relatively high prion titers [(D − N) > $10^4$ cpm; marked with asterisks] were used to prepare inocula for transmission experiments with Tg mice expressing cervid PrP. (B) CWD samples used for transmission experiments with Tg mice expressing HuPrP, BoPrP, or OvPrP.

**$PrP^{Sc}$ levels in the brains of elk and deer with CWD.** For transmission studies of CWD prions to Tg mice, we examined six brainstem samples from elk, five from mule deer, and six from white-tailed deer, all of which originated from confirmed CWD cases and contained $PrP^{Sc}$. Using the CDI, we determined that relative $PrP^{Sc}$ levels varied 10- to 100-fold among the 17 samples (Fig. 1) (37, 39). In addition, DNA sequencing

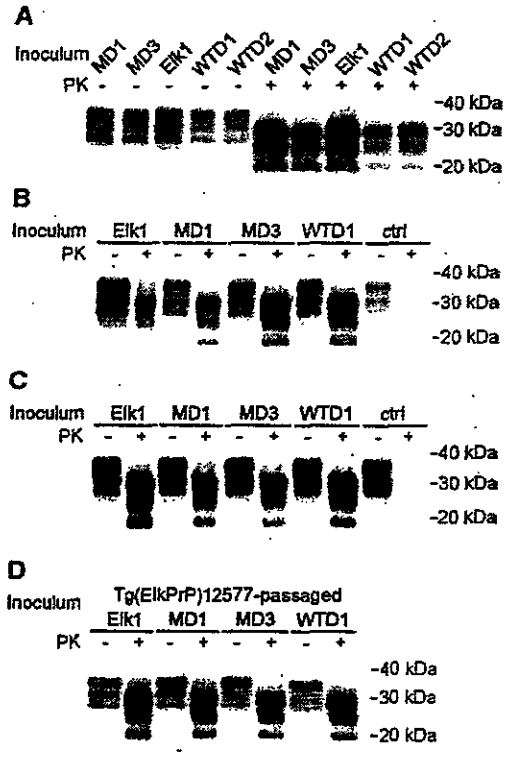


FIG. 2. Western blot analyses show that characteristics of CWD prions from diseased cervids remain similar after passage to Tg(DePrP) and Tg(ElkPrP) mice. (A) Brain homogenates of samples harboring high prion titers, as measured by the CDI and shown in Fig. 1. (B and C) Brain homogenates of diseased Tg(ElkPrP)12577 mice (B) and Tg(DePrP)10945 mice (C) infected with different CWD isolates after first passage, as indicated. ctrl, brain homogenate from an uninfected control mouse. (D) Brain homogenates of diseased Tg(ElkPrP)12577 upon second passage of different CWD isolates; first passage was in Tg(ElkPrP)12577 mice. In all panels, samples were either subjected to limited digestion with PK (+) or left undigested (−). Blots were probed using recFab HuM-P. Apparent molecular masses based on migration of protein standards are shown in kilodaltons.

of brain samples used to inoculate Tg mice expressing cervid PrP demonstrated that these samples were homozygous for *Prnp*. Among these, all elk samples had a PrP sequence identical to that expressed in Tg(ElkPrP) mice, and all mule deer and white-tailed deer samples expressed a PrP sequence identical to that expressed in Tg(DePrP) mice. Only CWD samples with high levels of $PrP^{Sc}$ were used as inocula for transmission studies to Tg mice expressing cervid PrP; these were Elk1, MD1, MD3, WTD1, and WTD2 (Fig. 1A and 2A).

**Transmission of CWD prions to Tg mice.** Inoculation of Tg(ElkPrP) and Tg(DePrP) mice resulted in transmission with all CWD isolates from elk, mule deer, and white-tailed deer (Table 2). In contrast, uninoculated Tg mice remained healthy for more than 600 days (Table 1). The Elk1 isolate caused disease in Tg(ElkPrP)12577 mice in 184 days (Table 2; Fig. 3A) and in Tg(DePrP)10945 mice in 329 days (Table 2; Fig. 4). First passage of isolates MD1 and MD3 into Tg(ElkPrP)12577 mice resulted in illness in 200 days (Table 2; Fig. 3B and C) and in Tg(DePrP)10945 mice in 339 and 292 days, respectively (Table 2; Fig. 4). For inoculation of isolates WTD1 (Fig. 3D) and WTD2, first passage in Tg(ElkPrP)12577 mice resulted in disease in 179 and 182 days, respectively (Table 2), and in Tg (DePrP)10945 mice in 408 and 399 days (Fig. 4), respectively. Western blot analysis showed identical $PrP^{Sc}$-banding patterns for all CWD inocula (Fig. 2A), which remained unchanged upon passage in Tg(ElkPrP)12577 mice (Fig. 2B) and Tg (DePrP)10945 mice (Fig. 2C). Brain homogenates prepared from CWD-inoculated Tg(ElkPrP)12577 and passaged into other Tg(ElkPrP)12577 mice also resulted in the same $PrP^{Sc}$-banding pattern (Fig. 2D). Additionally, quantification of the glycoforms in PK-resistant $PrP^{Sc}$ of the natural and mouse-passaged CWD prions confirmed that all samples had similar glycosylation ratios (see Fig. S1 in the supplemental material).

Mean incubation times for Tg(ElkPrP) lines with higher $PrP^{C}$ expression levels were shorter than for Tg(DePrP) lines (Table 2; Fig. 5). The level of ElkPrP and of DePrP was plotted as a function of the incubation time for the seven Tg cervid PrP lines inoculated with the Elk1 sample (Fig. 5). As shown, the length of the incubation time is inversely related to the level of expression of the cervid PrP transgene for the seven lines tested. This relationship is reminiscent of that found for Tg mice expressing Syrian hamster PrP (SHaPrP) (35).

**Incubation periods in Tg mice decreased upon second passage.** The second passage of Elk1, MD1, MD3, and WTD1 resulted in a shortening of the mean incubation periods by 10 to 70 days. For the second passage of each isolate, we prepared the inoculum from brain homogenates of individual Tg(ElkPrP)12577 mice that had been euthanized upon the onset of neurologic symptoms. Second passage of Elk1 prions reduced the mean incubation period from ~180 days to ~120 days and ~140 days (Table 3; Fig. 3A). The mean incubation period for the MD1 isolate decreased from 200 days to ~130 and ~140 days (Table 3; Fig. 3B), and that for the MD3 isolate decreased from 200 days to ~150 and ~190 days (Table 3; Fig. 3C). The second passage of the WTD1 isolate decreased the mean incubation period from ~180 days to ~150 and ~160 days (Table 3; Fig. 3D).

TABLE 2. Transmission of CWD prions to Tg mice expressing cervid PrP

| Transgenic line | Cervid PrP expression (*n*-fold) | Inoculum | Incubation time ± SEM (days) | No. of animals ill/no. of animals inoculated |
|---|---|---|---|---|
| Tg(ElkPrP)12577 | 2 | Elk1 | 184 ± 5.7 | 7/7 |
| | | MD1 | 200 ± 4.3 | 6/6 |
| | | MD3 | 200 ± 9.1 | 8/8 |
| | | WTD1 | 179 ± 9.1 | 7/7 |
| | | WTD2 | 182 ± 7.0 | 8/8 |
| Tg(ElkPrP)12580 | 2 | Elk1 | 204 ± 8.5 | 8/8 |
| Tg(ElkPrP)3934 | 1.5[a] | Elk1 | 264 ± 15.8 | 8/8 |
| Tg(ElkPrP$^{+/+}$)3934 | 3 | Elk1 | 145 ± 9.0 | 7/7 |
| Tg(ElkPrP)12584 | 3 | Elk1 | 149 ± 8.3 | 7/7 |
| Tg(DePrP)10945 | 1 | Elk1 | 329 ± 22.2 | 8/8 |
| | | MD1 | 339 ± 25.9 | 8/8 |
| | | MD3 | 292 ± 17.6 | 8/8 |
| | | WTD1 | 408 ± 30.8 | 6/6 |
| | | WTD2 | 399 ± 21.6 | 5/5 |
| Tg(DePrP)10969 | 1 | Elk1 | 304 ± 21.0 | 6/7 |
| | | MD1 | 323 ± 16.1 | 8/8 |

[a] Half of the level of ElkPrP expression in Tg(ElkPrP$^{+/+}$)3934 mice.

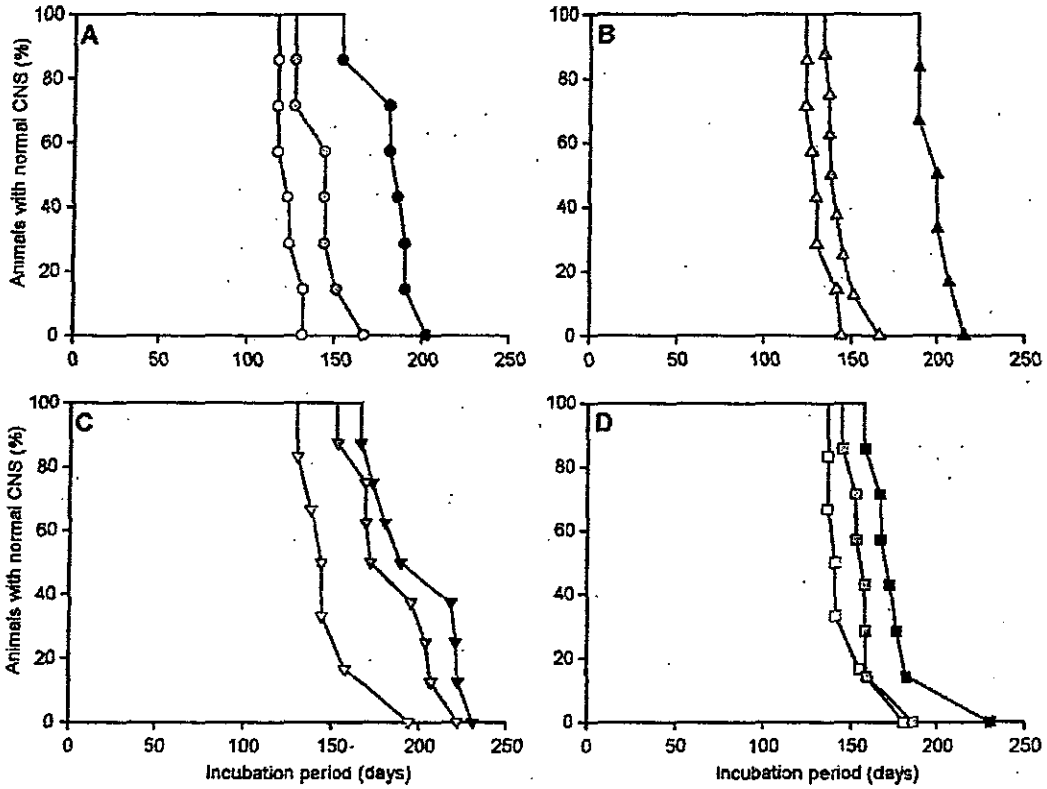


FIG. 3. Survival curves from transmissions of Elk1 (filled circles) (A), MD1 (filled triangles) (B), MD3 (filled inverted triangles) (C), and WTD1 (filled squares) (D) to Tg(ElkPrP)12577 mice. Serial transmission of 1% brain homogenates from Tg(ElkPrP)12577 mice that were diagnosed with clinical signs of prion disease after inoculation with the Elk1 isolate at 154 days (gray circles) and 181 days (open circles) (A), with the MD1 isolates at 189 days (two mice; gray and open triangles) (B), with the MD3 isolate at 166 days (gray triangles) and 173 days (open triangles) (C), and with the WTD1 isolate at 158 days (gray squares) and 167 days (open squares) (D).

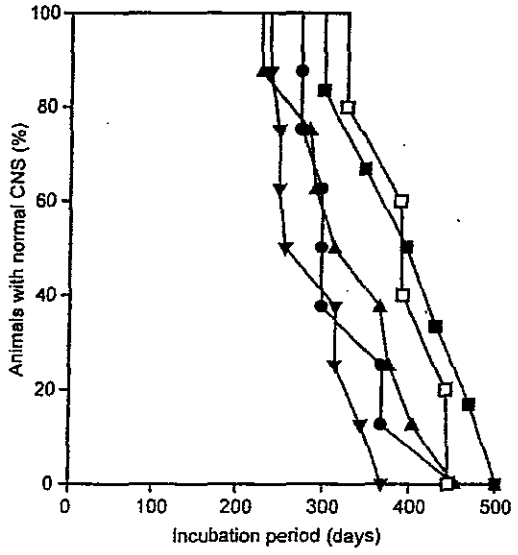


FIG. 4. Survival curves from transmissions of Elk1 (filled circles), MD1 (filled triangles), MD3 (filled inverted triangles), WTD1 (filled squares), and WTD2 (open squares) to Tg(DePrP)10945 mice.

**Neuropathology in Tg mice.** Histologic analysis showed that all CWD isolates caused similar neuropathologic changes in Tg(ElkPrP) and Tg(DePrP) mice, which did not vary upon second passage in Tg(ElkPrP)12577 mice. Immunohistochemistry with recFab HuM-P directed against PrP showed similar neuropathologic changes for all five isolates in the Tg(Elk

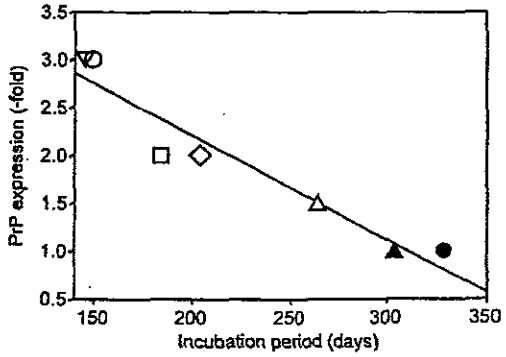


FIG. 5. Mean incubation periods in Tg mice expressing cervid PrP inoculated with Elk1 prions. The expression level of PrP inversely correlates with the incubation time ($R$ = 0.96). Open inverted triangle, Tg(ElkPrP$^{+/+}$)3934; open circle, Tg(ElkPrP)12584; open square, Tg(ElkPrP)12577; open diamond, Tg(ElkPrP)12580; open triangle, Tg(ElkPrP)3934; filled triangle, Tg(DePrP)10969; filled circle, Tg(DePrP)10945.

TABLE 3. Serial passage of CWD prions in Tg(ElkPrP)12577 mice

| Inoculum (day)[a] | Incubation time ± SEM (days) | No. of animals ill/no. of animals inoculated |
|---|---|---|
| Elk1 (181) | 123 ± 2.5 | 7/7 |
| Elk1 (154) | 143 ± 5.2 | 7/7 |
| MD1 (189)[b] | 131 ± 3.0 | 7/7 |
| MD1 (189)[b] | 144 ± 3.7 | 8/8 |
| MD3 (173) | 151 ± 9.3 | 6/6 |
| MD3 (166) | 186 ± 8.5 | 8/8 |
| WTD1 (167) | 149 ± 6.9 | 6/6 |
| WTD1 (158) | 159 ± 4.9 | 7/7 |

[a] All inocula shown were serially passaged into Tg(ElkPrP)12577 mice. The day on which the animal was sacrificed due to the onset of prion disease is given for each inoculum.

[b] These inocula were prepared from two individual animals that were diagnosed with illness at 189 days postinoculation.

PrP)12577 (Fig. 6A to C) and Tg(DePrP)10945 (Fig. 6D to F) mice. Numerous PrP amyloid plaques, common in CWD (3, 15, 55), were seen in the brains of ill Tg(ElkPrP) and Tg(DePrP) mice. Most plaques were periventricular, but some were present away from the ventricle, particularly in the hippocampal neuropil (Fig. 6A to F). Florid plaques were abundant (Fig. 6G), and staining with Thioflavine S verified that many PrP-positive plaques were true amyloid (Fig. 6H). Hematoxylin and eosin staining showed widespread spongiform degeneration within the hippocampus, while staining with antibodies against GFAP revealed intensive astrocytic gliosis (data not shown). Histoblot analysis showed that CWD prions from elk, mule deer, and white-tailed deer resulted in similar patterns of $PrP^{Sc}$ deposition in the brains of Tg(ElkPrP) (Fig. 7A to D) and Tg(DePrP) mice (Fig. 7E to H).

**Inoculation of Tg(HuPrP), Tg(BoPrP), and Tg(OvPrP) mice with CWD prions.** To test the susceptibility of humans, cattle, and sheep to CWD prions, we intracerebrally inoculated Tg mice that overexpress either human PrP [Tg(HuPrP)], bovine PrP [Tg(BoPrP)], sheep PrP [Tg(OvPrP)], or MoPrP [Tg(MoPrP)] (Table 4). The inocula used contained high $PrP^{Sc}$ titers as confirmed by CDI (Fig. 1B). Tg(HuPrP)440 mice express HuPrP at a level twofold greater than that found in human brain; when inoculated with sCJD prions, these mice succumb to disease in ~150 days (47). In contrast, Tg(HuPrP)440 mice remained healthy for >500 days after infection with each of four different CWD isolates from elk (Elk4, Elk5, Elk6, and Elk7), two from mule deer (MD4 and MD5), and two from white-tailed deer (WTD4 and WTD5) (Table 4). Similar results were obtained with Tg(BoPrP) mice expressing PrP at levels 10-fold higher than

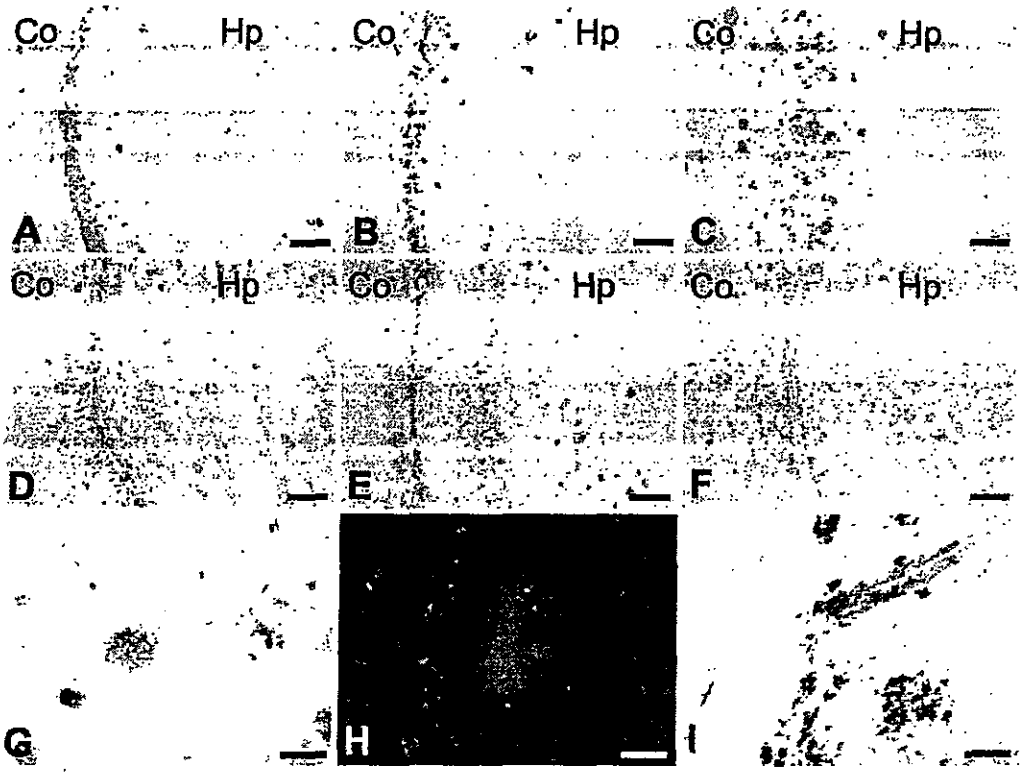


FIG. 6. Immunohistochemistry for $PrP^{Sc}$ with recFab HuM-P shows PrP amyloid plaque deposition in brain sections from Tg(ElkPrP)12577 mice (A to C), Tg(DePrP)10945 mice (D to H), and Tg(MoPrP)4053 mice (I) inoculated intracerebrally with CWD prions from elk (A, D, G, H, and I), mule deer (B and E), and white-tailed deer (C and F). In Tg(ElkPrP)12577 mice, most of the PrP plaques were located in the periventricular region with each of the CWD inocula (A to C). In Tg(DePrP)10945 mice, large numbers of PrP plaques were located both in the periventricular region and in the brain parenchyma away from the ventricles (D to F). Many florid plaques, characterized by a single $PrP^{Sc}$ deposit surrounded by vacuoles, were seen (G). Thioflavine S staining shows that many of the larger PrP plaque deposits are amyloid (H). Large deposits of PrP plaques were found in Tg(MoPrP)4053 mice following inoculation with elk CWD prions (I). Hp, Hippocampus; Co, Corpus callosum; bars in panels A to F, 150 μm; bars in panels G to I, 40 μm.

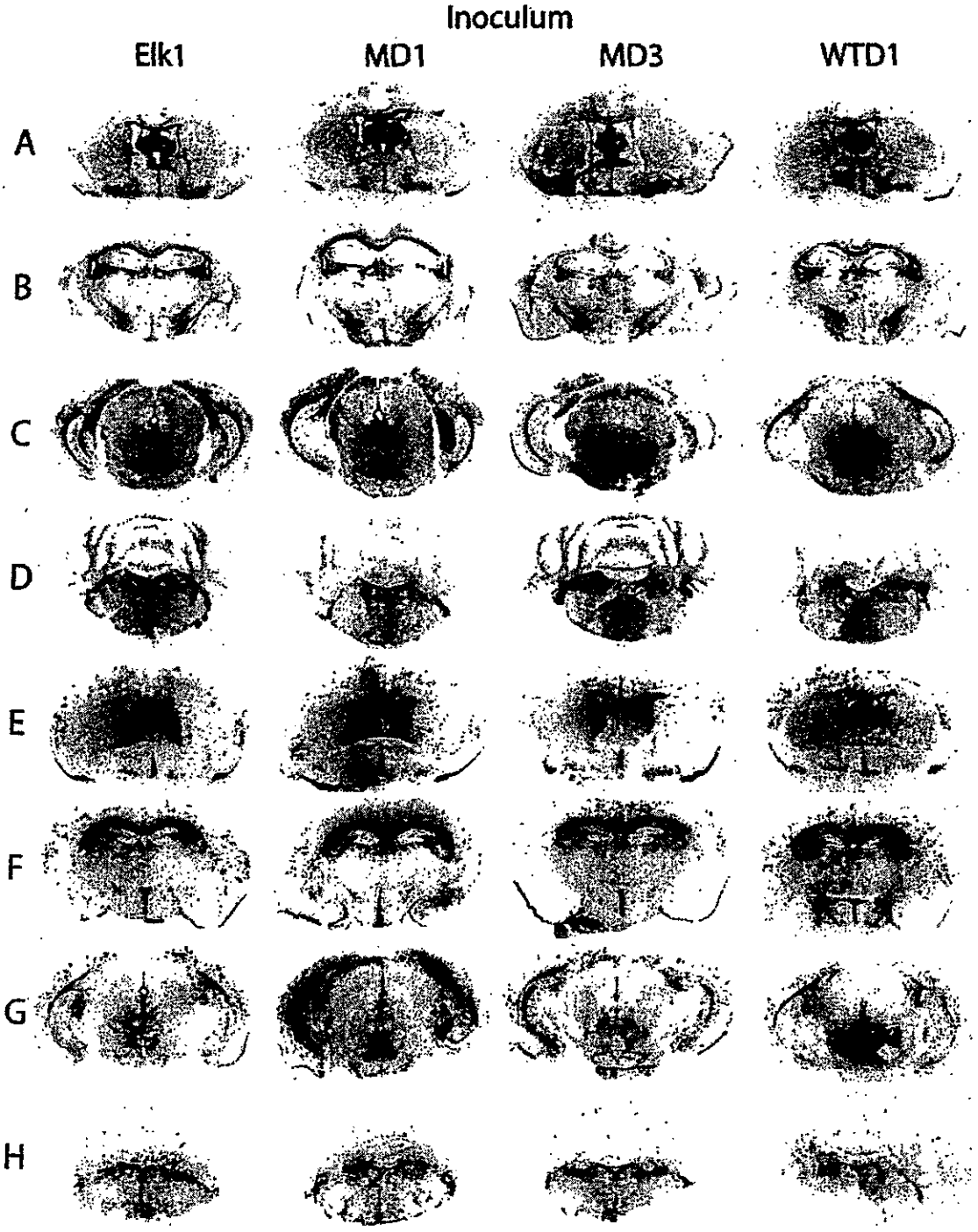


FIG. 7. Histoblot analysis shows the anatomic deposition of protease-resistant $PrP^{Sc}$ in Tg(ElkPrP)12577 (A to D) and Tg(DePrP)10945 (E to H) mice following inoculation with different CWD isolates, as indicated. Coronal sections from four different brain levels are shown: (A and E) head of the caudate nucleus; (B and F) dorsal hippocampus and thalamus; (C and G) midbrain; and (D and H) cerebellum and pons.

that found in cattle brain. When inoculated with BSE prions, Tg(BoPrP)4092 mice and Tg(BoPrP)4125 mice died of prion disease in ~240 days. In contrast, Tg(BoPrP)4092 mice remained healthy after inoculation with four CWD brain samples from elk (>600 days) and two samples each from mule deer and white-tailed deer (>500 days). Neither of the two elk samples inoculated into Tg(BoPrP)4125 mice resulted in prion disease (>600 days). Similarly, inoculation of three elk, two mule deer, and three white-tailed deer CWD samples into Tg(OvPrP,VRQ)338 mice did not transmit prion disease after >500 days. These Tg mice express OvPrP at a level 12-fold higher than that found in sheep

TABLE 4. Comparison of cervid prion inocula in Tg mice expressing human, bovine, ovine, and murine PrP genes

| Transgenic line | PrP expression (*n*-fold) | Inoculum | Incubation time ± SEM (days) | No. of animals ill/no. of animals inoculated[a] |
|---|---|---|---|---|
| Tg(HuPrP)440 | 2[b] | Elk4 | >540 | 0/11 A |
| | | Elk5 | >540 | 0/13 B |
| | | Elk6 | >532 | 0/8 |
| | | Elk7 | >538 | 0/7 |
| | | MD4 | >512 | 0/9 |
| | | MD5 | >512 | 0/6 |
| | | WTD4 | >512 | 0/5 |
| | | WTD5 | >539 | 0/8 |
| | | sCJD, M/M129 | 154 ± 2.6 | 18/18 |
| Tg(BoPrP)4092 | 10[c] | Elk4 | >622 | 0/6 |
| | | Elk5 | >600 | 0/11 C |
| | | Elk6 | >610 | 0/3 D |
| | | Elk7 | >610 | 0/6 |
| | | MD4 | >512 | 0/8 |
| | | MD5 | >532 | 0/8 E |
| | | WTD4 | >532 | 0/7 |
| | | WTD5 | >532 | 0/7 |
| | | BSE PG31/90 | 244 ± 4.8 | 9/9 |
| Tg(BoPrP)4125 | 8 to 16[d] | Elk4 | >610 | 0/10 F |
| | | Elk5 | >620 | 0/8 |
| | | BSE PG31/90 | 340 ± 5.3 | 41/41 |
| Tg(OvPrP,VRQ)338 | 12[e] | Elk4 | >516 | 0/5 G |
| | | Elk5 | >509 | 0/8 H |
| | | Elk6 | >509 | 0/11 |
| | | MD4 | >516 | 0/9 |
| | | MD5 | >516 | 0/11 |
| | | WTD4 | >505 | 0/8 |
| | | WTD5 | >505 | 0/11 I |
| | | WTD6 | >526 | 0/12 |
| | | Scrapie 027 | 178 ± 9.3 | 16/16 |
| Tg(MoPrP)4053 | 8[f] | Elk4 | 542 ± 32.1 | 6/8 |
| | | Elk5 | >590 | 0/10 |
| | | Elk6 | >512 | 0/7 J |
| | | Elk7 | >512 | 0/8 |
| | | MD4 | >539 | 0/5 |
| | | MD5 | >512 | 0/5 |
| | | WTD4 | >512 | 0/3 K |
| | | WTD5 | >532 | 0/10 |
| | | RML | 59 ± 1.5 | 10/10 |

[a] Animals with intercurrent illness at later stages of the experiments died on the indicated days postinoculation (unless otherwise noted, one animal died on the indicated day): A, 428 days; B, 481 days; C, 589 days; D, 588 days (two animals); E, 399 days; F, 384 days; G, 446 and 494 days; H, 232 days (two animals); I, 291 days; J, 518 days; K, 224 days. These animals were determined to be free of prion disease after neuropathologic analysis for spongiform degeneration and immunohistochemistry for PrP and GFAP.

[b] Compared to $PrP^C$ in human brain (47).

[c] Compared to $PrP^C$ in wild-type FVB mouse brain.

[d] Compared to $PrP^C$ in bovine brain (43).

[e] Compared to $PrP^C$ in sheep brain, listed as 6× for the hemizygous line Tg(OvPrP,$VRQ^{+/0}$)338 (26).

[f] Compared to $PrP^C$ in wild-type FVB mouse brain (46).

brain. When inoculated with sheep scrapie prions, the Tg(OvPrP,VRQ)338 mice developed neurologic dysfunction in <200 days. Occasional Tg mice expressing HuPrP, BoPrP, or OvPrP that had been inoculated with CWD prions died at an older age (Table 4). The brains of those mice were examined histologically for spongiform changes and immunohistochemically for PrP and GFAP staining. None of the Tg mice showed evidence of prion disease.

In contrast to Tg mice expressing human, bovine, or ovine PrP, Tg(MoPrP)4053 mice were susceptible to one CWD prion isolate, albeit after prolonged incubation periods (Table 4). Tg(MoPrP)4053 mice express wild-type MoPrP-A at eightfold-higher levels than WT FVB mice. Six of eight mice became ill after inoculation with the Elk4 CWD sample beginning at 460 days, with a mean incubation period of ~540 days. Inoculation with three other CWD samples from elk, two from mule deer, and two from white-tailed deer did not produce disease after >500 days. Neuropathologic examination of diseased Tg(MoPrP)4053 mouse brain showed spongiform degeneration, $PrP^{Sc}$ deposition, and PrP amyloid plaques (Fig. 6I).

## DISCUSSION

The overexpression of cervid PrPs in mice did not have any deleterious effect on the Tg lines described here. Uninoculated mice from one such Tg line was observed for ~650 days (Table 1). The absence of spontaneous disease in these Tg mice allowed us to use them to bioassay prions in the brains of elk and deer that died of CWD.

Brainstem samples from elk, mule deer, and white-tailed deer with CWD were inoculated into five Tg lines expressing ElkPrP and two lines expressing DePrP. Bioassay of the Elk1 inoculum in the seven Tg cervid PrP lines showed that the length of the incubation time is inversely proportional to the level of cervid PrP expression in the brain (Fig. 5; Table 2). When the level of cervid $PrP^C$ expression was similar to that of $MoPrP^C$ in WT mice, it was designated 1×. In Tg(DePrP) mice expressing DePrP at 1×, the incubation time was ~300 days, whether the CWD inoculum was from mule deer (MD1) or elk (Elk1). Doubling the level of cervid $PrP^C$ to 2× resulted in a reduction of the incubation time to ~200 days for the Elk1 and MD1 inocula while tripling the expression of cervid $PrP^C$ reduced the incubation time to ~100 days for the Elk1 inoculum. A similar relationship was described earlier for Tg mice expressing $SHaPrP^C$ (35); however, both MoPrP and SHaPrP were coexpressed in those Tg lines. In the studies reported here (Fig. 5), the MoPrP gene was disrupted (10) so that the only PrP being expressed was cervid $PrP^C$. In a recent study, Tg mice expressing DePrP at 5× and 3× the level of PrP expressed in WT FVB mice developed neurologic deficits at ~235 days after intracerebral inoculation with CWD prions from elk and at 225 to 264 days with CWD prions from mule deer (9). In another study, two lines of Tg mice expressing ElkPrP at 2× developed CNS disease 118 or 142 days after inoculation with CWD prions from elk (23).

The CDI studies of the CWD inocula indicated that the Elk1 and MD1 inocula contained similar levels of $PrP^{Sc}$ (Fig. 1A), which is consistent with the indistinguishable incubation times for these inocula in Tg(ElkPrP)12577, Tg(DePrP)10945, and Tg(DePrP)10969 mice (Table 2). Interestingly, the levels of $PrP^{Sc}$ varied over a >100-fold range for the first nine cervid brain specimens examined (Fig. 1A). Assessing the level of $PrP^{Sc}$ in brain samples in advance of our transmission studies proved to have been quite useful in retrospect (Table 2).

Serial passage of CWD prions in the Tg(ElkPrP) mice resulted in modest reductions in the incubation times, i.e., up to ~70 days (Fig. 3A to D; Table 3). This shortening was seen with prions from elk, mule deer, and white-tailed deer. These

results contrast with those for the serial passaging of BSE prions in Tg(BoPrP) mice and the serial passaging of CWD prions in Tg12 mice, for which no changes in the incubation times were observed (23, 42, 44). There are several possible explanations for the shortening of the incubation times upon serial passage in Tg(ElkPrP)12577 mice. First, the level of prions in the brains of cervids may be lower than in Tg(Elk PrP)12577 mice. If that were the case, then the first Tg mouse-to-Tg mouse passages would be expected to exhibit shorter incubation times than those found with passages from the cervids to Tg mice. A corollary to this scenario is that the incubation times upon subsequent passage in a given Tg line should remain constant. Second, the cervid brain inocula may be composed of a mixture of strains, and one strain may emerge as the predominant strain over the length of the incubation time. In this case, the predominant strain in the Tg mouse brain exhibits a shorter incubation time during the next passage, because it exists at a higher titer in the mouse brain than in the cervid brain sample. Third, within a mixture of prion strains, some slow strains may be inhibitory for faster ones as previously reported (13, 22). If this were the case and transmission to Tg mice resulted in the elimination of the slower strain, then on subsequent passage in Tg mice, the incubation time would shorten. Fourth, a posttranslational modification in cervid PrP, such as the N-linked oligosaccharides or the glycosylphosphatidylinositol anchor, might slow replication of cervid prions in the Tg mice. If this were the case, then on subsequent passage, CWD prions formed in a mouse would exhibit shorter incubation times.

Except for the first possibility, for which endpoint titrations can be used to establish the titers of CWD prions in cervid and Tg mouse brains, distinguishing among the possibilities listed above may be difficult. Interpreting such a titration study will be facilitated if endpoint titrations in cervids give results similar to those obtained with the Tg mice. It is notable that endpoint titrations performed with cattle resulted in a titer of $10^6$ 50% infective dose units/g of brain tissue from the obexes of BSE-infected cattle, whereas endpoint titrations performed with Tg(BoPrP) mice resulted in a titer of $10^{6.9}$ 50% infective dose units/g of brain tissue (39, 42, 50).

Both the glycoform abundance patterns and the distribution of neuropathologic lesions in CWD-inoculated Tg(ElkPrP) and Tg(DePrP) mice argue for a single prion strain. The molecular masses of the di-, mono-, and unglycosylated $PrP^{Sc}$ fragments from all CWD isolates were similar before (Fig. 2A) and after passaging in Tg(ElkPrP)12577 (Fig. 2B and D) and Tg(DePrP)10945 (Fig. 2C) mice. Moreover, the relative abundance of these glycoforms did not change upon passage in the Tg mice (see Fig. S1 in the supplemental material).

Neuropathologic examination of ill Tg(ElkPrP) and Tg(DePrP) mice demonstrated similar CNS lesions in all of the mice inoculated with CWD prions from elk, mule deer, and white-tailed deer (Fig. 6). The deposition patterns and neuroanatomic distribution of both $PrP^{Sc}$ deposition (Fig. 7) and florid PrP amyloid plaques (Fig. 6) were similar for all inocula but differed somewhat in intensity. Upon serial passage in Tg(Elk PrP) mice, the CNS lesions remained unchanged (data not shown). Overall, our neuropathologic findings for CWD-infected Tg mice expressing DePrP or ElkPrP did not differ substantially from those reported by others (9, 23).

In Tg(MoPrP)4053 mice inoculated with CWD prions, both the morphology of the lesions and distribution of PrP amyloid plaques (Fig. 6I) were different from those found in Tg(Elk PrP) and Tg(DePrP) mice. In contrast to RML prions that produce finely granular deposits of $PrP^{Sc}$ in the absence of amyloid plaques, the CWD prion strain was amyloidogenic in the brains of Tg(MoPrP)4053 mice.

In contrast to Tg mice expressing cervid PrP, Tg mice expressing human, bovine, or ovine PrP did not succumb to prion disease after inoculation with CWD prions after >500 days (Table 4). Our results agree with those of others who reported that Tg mice expressing human PrP were resistant to CWD prions but susceptible to sCJD prions (23). Despite our confirmation of an earlier study demonstrating that Tg mice expressing HuPrP(M129) do not develop prion disease when inoculated with CWD prions, any conclusions from such negative data need to be tempered (7), especially in light of a recent study with squirrel monkeys. Intracerebral inoculation of CWD prions into squirrel monkeys (*Saimiri sciureus*) demonstrated transmission to a nonhuman primate, arguing that humans might be susceptible to CWD prions (27).

While our studies of Tg(BoPrP) mice inoculated with CWD prions also gave negative results, a recent study reported that five of 13 cattle inoculated with CWD prions produced $PrP^{Sc}$, based on limited proteolysis and immunohistochemical staining of brain sections (16). These studies were terminated 6 years after intracerebral inoculation, before any of the cattle developed neurologic dysfunction. In other studies, sheep scrapie prions were inoculated into elk (17). After more than three years, $PrP^{Sc}$ was found in the brains of three elk that developed neurologic deficits before dying. Our negative results with CWD prions inoculated into Tg(HuPrP), Tg(Bo PrP), and Tg(OvPrP) mice might reflect low levels of prion replication that are insufficient to produce disease during the 500-day observation period. Several investigators have described situations in which prions from one species replicate too slowly in another species to cause signs of neurologic dysfunction but do produce disease with serial passage (19, 36). In the studies reported here, we did not choose to passage serially the brains of asymptomatic, CWD-inoculated Tg(Hu PrP), Tg(BoPrP), and Tg(OvPrP) mice (Table 4). An alternative explanation for our negative results may reside in the strain(s) of CWD prions that we used for inoculation. While our CWD prions were unable to initiate the conversion of $HuPrP^C$, $BoPrP^C$, or $OvPrP^C$ into $PrP^{Sc}$, some other CWD strains might be able to do so. Precedent for the latter has been seen with human prions: human vCJD prions replicate well in Tg(BoPrP) mice but multiply slowly in Tg(HuPrP) mice and in Tg(MHu2M) mice expressing a chimeric human-mouse PrP (2, 24, 42, 44).

Whether hunters, cervid farmers, and aficionados of venison are at increased risk for prion disease remains to be established. Recently, CWD prions were also reported in the skeletal muscles of infected deer, indicating a possible hazard for the oral transmission of CWD prions (1). Tg(ElkPrP) and Tg(DePrP) mice provide a sensitive and specific bioassay for determining the levels of CWD prion infectivity in cervid tissues and for studying the biology of these particular prions. These Tg mice make it possible to determine the levels of CWD prion titers in brain, muscle, liver, pancreas, kidney, and

other tissues as well as in the blood, urine, feces, and saliva of both elk and deer. Elucidating the mode of CWD prion spread among elk, deer, and moose will be important for understanding why CWD prions are so contagious for domesticated and free-ranging cervids. Such information may prove useful in learning how to restrict the epizootic spread of CWD prions to humans and livestock.

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**研究報告の概要**

○輸血に伴う変異型クロイツフェルト・ヤコブ病の臨床像および生前診断:症例報告

【背景】変異型クロイツフェルト・ヤコブ病(vCJD)が輸血によって感染する可能性があるという懸念が浮上している。vCJDを発症した供血者の血液を輸血されたことが判明している集団から、プリオン感染症2例が死後に特定され、2004年に報告された。このリスク群の別の1名の患者は、神経学的徴候を発現し、National Prion Clinicへ照会された。

【方法】この1名の患者は検査のため入院となり、National Blood ServiceおよびHealth Protection Agencyから輸血歴の詳細が得られた。当該患者はvCJDと診断された後、MRC PRION-1 trialに登録された。患者が死亡した際、剖検時に脳と扁桃腺の組織を得、免疫ブロッティング法および免疫組織化学検査により異常プリオンの存在を調べた。

【知見】vCJDであることがほぼ確定という臨床診断が下された。扁桃腺の生検は実施しなかった。当該患者は、キナクリンを用いた実験的治療を受けたが、容体は悪化し、典型的なvCJDの臨床経過を経て死亡した。剖検により診断が確認され、扁桃腺のプリオン感染が示された。

【解釈】死亡前に特定された当該輸血関連vCJD症例は、後にvCJDを発症した供血者から輸血を受けて5年以上生存した受血者集団23名からの3例目の患者である。この輸血を受けた残りの受血者のリスクは高いと考えられるため、専門医のフォローアップと検査を受けるべきである。扁桃腺の生検は、ウシ海綿状脳症プリオンの1次感染患者と同様、医原的曝露を被った他の高リスク患者においても、早期の症状発現前診断を可能にする。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 英国で3例目の輸血関連変異型クロイツフェルト・ヤコブ病の症例報告である。 | 日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ケ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。 |

# Clinical presentation and pre-mortem diagnosis of variant Creutzfeldt-Jakob disease associated with blood transfusion: a case report

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## Summary

**Background** Concerns have been raised that variant Creutzfeldt-Jakob disease (vCJD) might be transmissible by blood transfusion. Two cases of prion infection in a group of known recipients of transfusion from donors who subsequently developed vCJD were identified post-mortem and reported in 2004. Another patient from this at-risk group developed neurological signs and was referred to the National Prion Clinic.

**Methods** The patient was admitted for investigation and details of blood transfusion history were obtained from the National Blood Service and Health Protection Agency; after diagnosis of vCJD, the patient was enrolled into the MRC PRION-1 trial. When the patient died, brain and tonsil tissue were obtained at autopsy and assessed for the presence of disease-related PrP by immunoblotting and immunohistochemistry.

**Findings** A clinical diagnosis of probable vCJD was made; tonsil biopsy was not done. The patient received experimental therapy with quinacrine, but deteriorated and died after a clinical course typical of vCJD. Autopsy confirmed the diagnosis and showed prion infection of the tonsils.

**Interpretation** This case of transfusion-associated vCJD infection, identified ante-mortem, is the third instance from a group of 23 known recipients who survived at least 5 years after receiving a transfusion from donors who subsequently developed vCJD. The risk to the remaining recipients of such tranfusions is probably high, and these patients should be offered specialist follow-up and investigation. Tonsil biopsy will allow early and pre-symptomatic diagnosis in other iatrogenically exposed individuals at high risk, as in those with primary infection with bovine spongiform encephalopathy prions.

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See Comment page 2037

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## Introduction

Since the arrival of variant Creutzfeldt-Jakob disease (vCJD), and the experimental confirmation that it is caused by the same prion strain as that causing epidemic bovine spongiform encephalopathy (BSE), there have been concerns that it might be transmissible by iatrogenic routes including use of blood and blood products.[1] Such concerns were heightened by the recognition that the pathogenesis of vCJD differed substantially from that of sporadic or classical CJD by showing prominent and uniform prion accumulation in lymphoreticular tissues[2,3] akin to ovine scrapie, where prionaemia has since been demonstrated experimentally.[4] All clinically affected patients with vCJD identified to date have been homozygous for methionine at polymorphic codon 129 of the prion protein (PrP) gene, *PRNP*, where either methionine (M) or valine (V) may be encoded.

Several risk reduction measures have been introduced in the UK and some other countries to limit secondary transmission of vCJD prions by blood and surgical instruments. In 1997, a study was set up between the National CJD Surveillance Unit and UK Blood Services so that recipients of blood components from donors who subsequently developed vCJD (ie, vCJD-implicated components) could be identified and evidence sought for possible transmission of vCJD by blood transfusion.[5]

In 2004, two transfusion-associated cases of vCJD prion infection were reported in individuals who had been identified in this way. The first was in a 62-year-old patient who had received a single vCJD-implicated non-leucodepleted unit of red cells and who became symptomatic 6·5 years post-transfusion and died after a progressive neurological illness of estimated 13 months' duration. The pulvinar sign was not seen on brain MRI and tonsil biopsy was not done. The cause of death was recorded as dementia.[5] After autopsy, neuropathology was thought to be suggestive of CJD, and vCJD was subsequently confirmed by neuropathological examination; the clinical presentation of vCJD in the case was judged typical of vCJD.[5]

The second case was in an elderly patient who had also received a single vCJD-implicated unit of non-leucodepleted red cells and who died from an unrelated cause, without evidence of a neurological disorder, 5 years after receiving the transfusion. Western blot analysis for the disease-related prion protein isoform ($PrP^{Sc}$) and PrP immunohistochemistry of lymphoreticular tissues was consistent with vCJD prion infection.[6] Interestingly, this patient was heterozygous (MV) at *PRNP* codon 129, a genotype that is associated with relative resistance to CJD and other prion diseases.[7] The cohort of identified recipients of blood transfusion from donors who

For UK Blood Services see http://www.cjd.ed.ac.uk/TMER/TMER.htm

subsequently developed vCJD totals 66 individuals, of whom 42 have died (including both cases described above and the patient reported here) and 24 are alive. Since December, 2003, the surviving recipients and their doctors have been notified of their exposure to a vCJD-implicated transfusion. We report the development of vCJD in an individual from this cohort and discuss the clinical, neuroradiological, and pathological findings and their implications.

## Methods

### Research ethics and consent

The Medical Research Council (MRC) PRION-1 trial was established at the request of the UK Chief Medical Officer, and a national referral protocol was agreed by the MRC, Department of Health, the National Prion Clinic, and the National CJD Surveillance Unit so that all UK patients would have the opportunity to participate. The Chief Medical Officer wrote to all UK neurologists in 2004, requesting joint referral of all suspect cases to both the National CJD Surveillance Unit and the National Prion Clinic for possible participation in therapeutic trials and related research. The PRION-1 trial received ethics approval from the Eastern multi-centre research ethics committee. Tissues were removed at autopsy with consent for both diagnosis and research. Research studies on human tissue were approved by the Institute of Neurology/National Hospital for Neurology and Neurosurgery local research ethics committee.

### Immunoblotting

All procedures were done in a microbiological containment level-3 facility with strict adherence to safety protocols. Brain frontal cortex and tonsil lymphoid tissue were prepared as 10% weight per volume homogenates in Dulbecco's sterile phosphate buffered saline without calcium or magnesium using Duall tissue grinders (Anachem, Luton, UK).[3] Brain homogenates were analysed before or after proteinase K digestion (50 μg/mL final protease concentration, 1 h, 37°C) by immunoblotting with anti-PrP monoclonal antibody 3F4,[8] using high sensitivity enhanced chemiluminescence or standard enhanced chemifluorescence as described previously.[3] Tonsil homogenate was analysed by sodium phosphotungstic acid precipitation of $PrP^{Sc}$, proteinase K digestion, and immunoblotting with anti-PrP monoclonal antibody 3F4 using high sensitivity enhanced chemiluminescence as described previously.[3] This method facilitates highly efficient recovery and detection of $PrP^{Sc}$ from vCJD tissue homogenate when present at concentrations $10^4$–$10^5$ times lower than those found in vCJD brain.[3,9]

### Immunohistochemistry

Brain and tonsil were assessed with anti-PrP monoclonal antibody ICSM 35 (D-Gen, London, UK), using a Ventana automated immunohistochemical staining machine (Ventana Medical Systems, Tucson, AZ, USA) as described previously.[9] Tissue was fixed in 10% buffered formal saline followed by incubation in 98% formic acid for 1 h. Following further washing for 24 h in 10% buffered formal saline, tissue samples were processed and embedded in paraffin wax. Sections were cut at a nominal thickness of 4 μm, treated with 98% formic acid for 5 min and then boiled in a low ionic strength buffer (2·1 mM Tris, 1·3 mM EDTA, 1·1 mM sodium citrate, pH 7·8) for 20 min. Abnormal PrP accumulation was examined using anti-PrP monoclonal antibody ICSM 35 followed by a biotinylated-anti-mouse IgG secondary antibody (iView Biotinylated Ig, Ventana Medical Systems) and an avidin-biotin horseradish peroxidase conjugate (iView SA-HRP, Ventana Medical System) before development with 3'3 diaminobenzedine tetrachloride as the chromogen (iView DAB, Ventana Medical Systems). Haematoxylin was used as the counterstain. Haematoxylin and eosin staining of serial sections was done with conventional methods. Appropriate controls were used throughout.

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

## Results

A 31-year-old male patient presented to the National Prion Clinic in 2005, with a 6-month history of progressive balance difficulties and impaired concentration. He had incapacitating leg pains and increasing difficulty walking. The patient had suffered from ulcerative colitis since his teenage years. A severe relapse of symptoms at age 22 years had led to surgical intervention with a colectomy and ileostomy. In 1997 (age 23 years), the ileostomy had been converted to an ileoanal J-pouch. Complications in the immediate postoperative period included pelvic bleeding: the patient was transfused with 22 units of red cells, 15 units of fresh frozen plasma, and three platelet doses, and required a further laparotomy and intensive care management. He then made a good recovery. One of the donors of the non-leucodepleted red cells received by the patient developed vCJD 20 months after donating this blood. This donor was homozygous (MM) at *PRNP* codon 129 and died after an 11-month illness, with neuropathological confirmation of vCJD.

6 years after the vCJD-implicated transfusion (age 29 years), the patient had presented to his family doctor with a short history of fluctuating exertional fatigue and impaired concentration. This problem was presumed to have followed a viral upper respiratory tract infection. Symptoms fluctuated, and around this time the patient and his doctor were notified of his exposure to a vCJD-implicated transfusion in 1997, and that he was

considered to be at risk of vCJD. Neurological examination, including clinical cognitive assessment, was normal and no biological symptoms of depressive illness were noted. Routine electroencepahlogram and MR neuroimaging were normal (figure 1A and 1B). Some symptomatic improvement occurred, sufficient for the patient to return to work. At neurological review after a further year, persistent symptoms of fatigue and impaired concentration were noted with no abnormal examination findings.

6 months later (age 31 years), 7·5 years after the vCJD-implicated transfusion, progressive neurological symptoms led to referral to the National Prion Clinic. The patient reported a sense of imbalance on turning with a tendency to stagger. Memory problems were becoming more prominent and he reported difficulties in recalling details of events in the preceding few days. He described a sense of tremor in his hands with reduced manual dexterity. Within a few weeks he reported severely incapacitating leg pains as an unvarying "searing pain" worsened by activity and eased by rest. Although most prominent in the anterior thighs, this pain affected the whole of both legs with hypersensitivity and paraesthesiae on touching the feet but no numbness or sensory loss. Examination 6 months after the onset of progressive neurological symptoms showed an mini mental state examination (MMSE) score of 30/30, digit recall of 6 forward and 5 backwards, with no evidence of dyspraxia or visuospatial dysfunction. The patient could walk unaided but preferred to use a stick to ease his leg pains. Other than minimal first-degree gaze-evoked nystagmus, cranial nerve and other neurological examination was normal.

Neurological decline continued with more prominent cognitive impairment. 6 weeks later, the patient's MMSE scored 22/30 (with errors in orientation for time, delayed recall, and serial subtraction). He was increasingly unsteady. Caregivers reported several falls, and the patient indicated that limb pains were less severe. Results of routine haematological and biochemical investigations, infection screen, and nerve conduction studies were normal. At this stage, MR neuroimaging showed symmetrical abnormal increased T2 and fluid attentuation inversion recovery (FLAIR) high signal within the posteromedial thalamus, consistent with the pulvinar sign (figure 1, C and D). Non-specific changes were evident on electroencepahlogram. *PRNP* genotyping excluded genetic mutations associated with inherited prion disease and showed codon 129 methionine homozygosity. Tonsil biopsy was declined. The patient elected to join the quinacrine arm of the MRC PRION-1 trial[10] and began treatment with quinacrine at 300 mg daily.

Subsequently the patient continued to decline with progressive cognitive impairment, ataxia, and dysarthria. He became bed-bound and poorly responsive before admission for hospice care. In line with the expressed wishes of the patient and his family, medical intervention was kept to a minimum and neither gastrostomy nor nasogastric feeding were instituted. The patient died 8 years and 8 months after the vCJD implicated transfusion (age 32 years) and an autopsy was done.

Brain and tonsil tissue were collected at autopsy and assessed for the presence of disease-related PrP by immunoblotting and immunohistochemistry. Immuno-

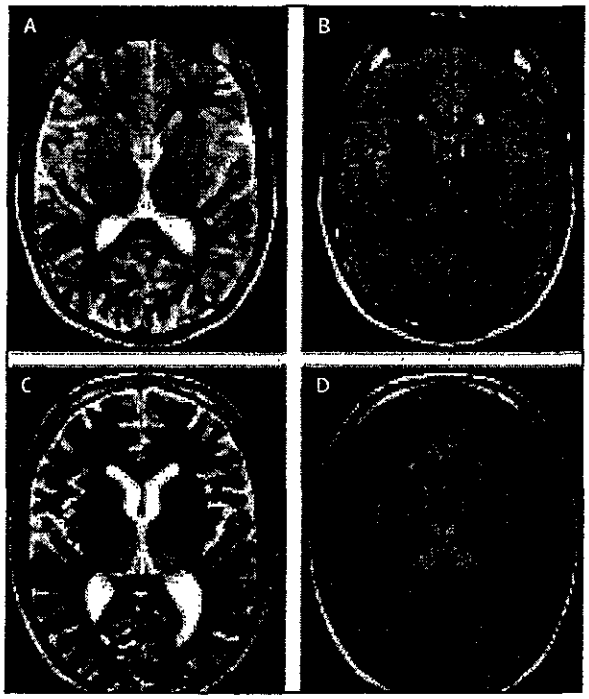


**Figure 1: MRI changes shown late in clinical course**
MR neuroimaging 6 years after vCJD-implicated transfusion showing normal appearances on T2 and FLAIR sequences (A, B), with abnormal increased T2 and FLAIR high signal within the posteromedial thalamus evident 18 months later (C, D).

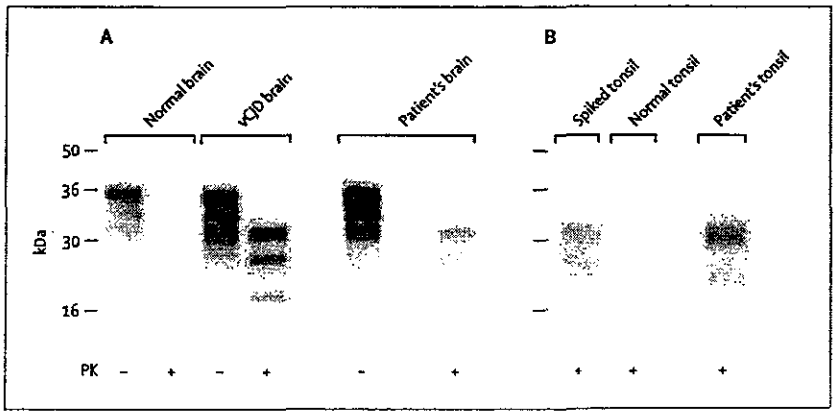


**Figure 2: Immunoblot detection of $PrP^{Sc}$ in brain and tonsil**
(A) 5-µl samples of 10% brain homogenate (frontal cortex) from normal human control, vCJD case, or the patient were analysed before (–) or after (+) proteinase K (PK) digestion. (B) PK-digested sodium phosphotungstic acid pellets from 0·5 mL 10% normal human tonsil homogenate or 0·5 mL 10% normal human tonsil homogenate spiked with 1 µL 10% brain homogenate from patient are compared with PK-digested sodium phosphotungstic acid pellet from 0·25 mL 10% tonsil homogenate from patient. Immunoblots were analysed with anti-PrP monoclonal antibody 3F4 using high sensitivity chemiluminescence.

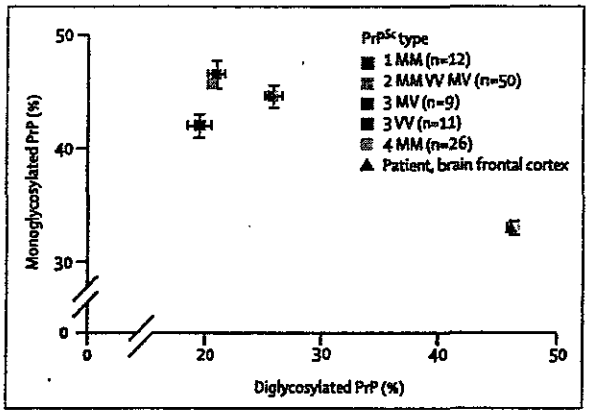


*Figure 3:* **Analysis of $PrP^{Sc}$ glycoform ratios**
Relative proportions of diglycosylated and monoglycosylated $PrP^{Sc}$ glycoforms after part-digestion of brain homogenate with proteinase K and immunoblotting with monoclonal antibody 3F4 using enhanced chemifluorescence. Key shows control cases of known $PrP^{Sc}$ type and *PRNP* codon 129 genotype. $PrP^{Sc}$ types 1–3 are seen in the brain in classical CJD (either sporadic or iatrogenic), $PrP^{Sc}$ type 4 is uniquely seen in vCJD brain.[11,12] $PrP^{Sc}$ typing according to classification of Hill et al.[11] Error bars show SE and where not visible were smaller than symbols used to designate means.

blotting of frontal cortex showed the presence of type 4 $PrP^{Sc}$ (figure 2A, figure 3) pathognomic of vCJD.[11] Immunohistochemical analysis of brain showed widespread deposition of abnormal PrP in all cortical areas and throughout the cerebellum, with occurrence of abundant florid PrP plaques (figure 4A and 4B) characteristic of vCJD.[13] Spongiform change, gliosis, and distribution of $PrP^{Sc}$ were indistinguishable from those found in previous cases of vCJD. Immunoblot analysis of lymphoid tissue from tonsil showed the presence of type 4t $PrP^{Sc}$,[2] at a concentration of about 1% of the level of $PrP^{Sc}$ detected in brain (figure 2B). Immunohistochemical analysis of tonsil showed multiple lymphatic follicles containing an abnormal PrP immunostaining pattern typical of that seen in vCJD (figure 4C).[14] Spleen was also positive (data not shown).

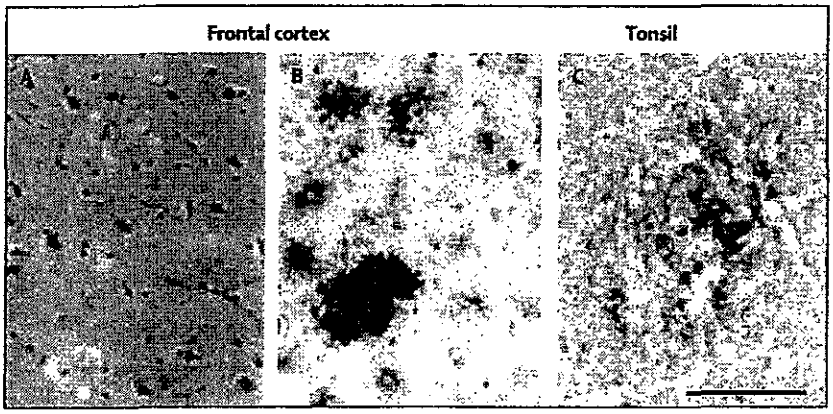


*Figure 4:* **PrP immunohistochemistry of patient's brain and tonsil**
(A) Haematoxylin and eosin staining of frontal cortex tissue showing florid plaques. (B) Immunohistochemistry of frontal cortex tissue showing abnormal PrP immunoreactivity, including abundant PrP plaques (anti-PrP monoclonal antibody ICSM 35). (C) Abnormal PrP immunoreactivity in tonsil (ICSM 35). The immunostaining pattern is typical of that observed in vCJD tonsil.[2,9] Scale bar=100 μm.

## Discussion

This third reported case of prion infection after red-cell transfusion from an asymptomatic donor who later developed vCJD emphasises the substantial risk faced by other recipients of vCJD-implicated blood components, and indeed suggests that blood transfusion is an efficient route of transmission of vCJD prion infection. The probability of vCJD occurring in a single individual in this at-risk population in the absence of transfusion-transmitted infection has been estimated at between one in 15 000 and one in 30 000;[5] the probability of chance occurrence of three such cases in this small group is remote. Uncertainty surrounds the risk of vCJD transmission by blood transfusion, and by products made by plasma fractionation, which have not yet been associated with any vCJD cases. Because the clinically silent incubation period of prion infections in human beings is known to be long (it can exceed half a century[15]), and the prevalence of asymptomatic infection in the general population of the UK is therefore unknown,[1] the extent of transfusion-transmission of vCJD can not yet be estimated. In the present and previously reported clinical cases, symptoms developed 6 and 6·5 years after blood transfusion, respectively; both individuals were homozygous for methionine (MM) at *PRNP* codon 129. Transmission of prion diseases between species are generally associated with much longer mean incubation periods than within-species transmissions, because of the so-called species or transmission barrier effect.[1] Therefore, mean incubation periods of secondary vCJD (involving person-to-person transmission) would be expected to have considerably shorter mean incubation periods than in primary vCJD resulting from exposure to BSE prions. That the incubation periods seen in the first cases of secondary vCJD, by definition those with the shortest incubation periods, were 6–6·5 years suggests that the shortest incubation periods of primary vCJD are longer, especially as the oral route of transmission is also typically associated with longer incubation periods than parenteral routes. A preliminary estimate of the shortest incubation periods of primary vCJD, based on the youngest cases seen, is around 12 years, which would be consistent with such extrapolation from the shortest incubation periods in secondary vCJD.[15]

The patient whose case we report here had ulcerative colitis. Experimentally induced chronic inflammatory foci can act as sites for prion propagation.[16] Although ulcerative colitis is a relatively common condition and not infrequently necessitates blood transfusion, it will be of interest to see if future cases had co-existent chronic inflammatory conditions at the time of prion exposure, which might indicate a role in susceptibility or incubation periods.

Whether individuals with the other *PRNP* codon 129 genotypes, VV and MV, will also succumb but with longer mean incubation periods is unknown. The *PRNP* 129MV blood recipient had evidence of prion

infection of lymphoreticular tissues at autopsy but died from a ruptured abdominal aortic aneurysm with no reported evidence of a neurological disorder; the brain showed no pathological features of vCJD.[6] It is unclear whether this individual would have gone on to develop clinical prion disease had they lived longer, and if so whether they would have had the phenotype of vCJD. Subclinical or carrier states of prion infection are recognised in animal models,[17,18] including BSE or vCJD prion-inoculated transgenic mice expressing human prion proteins.[19–21] Evidence from these studies suggests that individuals with such genotypes will be susceptible, but might develop disease phenotypes distinct from that of vCJD because of selection and propagation of different prion strain types.[20,22] The absence of detectable brain $PrP^{Sc}$ in the MV patient precluded molecular strain typing of prions. Although Peden and colleagues showed that splenic $PrP^{Sc}$ from this case resembled that seen in vCJD,[6] this demonstration required use of phosphotungstic acid precipitation of $PrP^{Sc}$, which interferes with molecular strain typing.[3] Additionally, type 5 $PrP^{Sc}$, seen in vCJD-inoculated transgenic mice expressing human PrP 129V, but not yet documented in humans, has a similar glycoform profile.[12,20,23] This patient might have been propagating a prion strain distinct from that causing vCJD.

Importantly, Peden and colleagues noted that they were unable to detect $PrP^{Sc}$ in lymphoid follicles in tonsil, appendix, or large intestine from their patient, although abnormal PrP was detected in spleen and in a cervical lymph node. Although concentrations of $PrP^{Sc}$ seen in their case were low,[6] perhaps indicating an early stage of incubation, $PrP^{Sc}$ levels are generally higher in tonsil than elsewhere in the lymphoreticular system in vCJD.[2,3] Because tonsillar involvement has to date been invariably seen in clinically affected cases of vCJD, Peden and colleagues speculated that this case might represent a distinctive pathogenesis related to route of exposure (intravenous rather than the oral route of exposure to BSE prions presumed in primary vCJD), and that the absence of a species barrier in secondary (person-to-person) infection might also affect phenotype. The current findings argue against these interpretations, and suggest that absence of tonsillar $PrP^{Sc}$ in the MV blood recipient might have been due to the effect of *PRNP* genotype and perhaps selection of a distinctive prion strain.

The patient's clinical course was characterised by a prolonged prodromal illness with fluctuating fatigue and clouded thinking, preceding progressive neurological deficit by 2 years. This case illustrates a clinical course of human vCJD prion infection characterised by five stages. A preclinical incubation phase of about 6 years occurred after blood transfusion, followed by a prodromal phase with fluctuating exertional fatigue and impaired concentration lasting about 18 months, and during which routine clinical examination and MR neuroimaging remained normal. Progressive neurological decline then consisted of an approximately 9-month initial phase of progressive ataxia, impaired manual dexterity, and dysaesthetic limb pain with substantial cognitive impairment but retained communication and comprehension of routine daily activities. A late neurological phase with severe cognitive decline and impaired verbal communication of about 4 months was followed by a shorter terminal phase in which the patient was bed-bound, mute, and only partly responsive.

Route of infection with prions is known to affect clinical phenotype[7] and, if directly related to prion infection, the lengthy fluctuating prodrome in this case might be a consequence of infection through blood transfusion. The early symptoms of vCJD are usually reported as non-specific, and detailed neurological examination in this patient at an unusually early stage in the clinical disease course was normal. The subsequent neurological decline characterised by progressive ataxia, cognitive impairment, and painful limb symptoms, was typical of vCJD.

vCJD has an insidious clinical onset, and its early features—such as depression, anxiety, personality change, and sensory disturbances—are highly non-specific. Early diagnosis is therefore difficult in the absence of another reason to suspect prion infection. This case illustrates the importance of diagnosis as early as possible in the clinical course. The patient was able to consider the option of joining a therapeutic clinical trial, to fully discuss the implications of his diagnosis, and to communicate his wishes to the clinical team and his family, making decisions and arrangements about his future care and other affairs. Specialist monitoring and investigation of high-risk groups, such as recipients of blood transfusion from a donor who developed vCJD, should allow the condition to be recognised at an early stage.

Careful clinical assessment remains the most important element in the early detection of symptomatic disease. The most useful non-invasive investigation in advanced cases of vCJD has been MR neuroimaging, particularly the FLAIR sequence.[24] Early case reports noted bilateral increased signal in the posterior thalamus (pulvinar) on T2 weighted images.[25] A retrospective review of MR scans from 36 histologically confirmed cases of vCJD, with a control group composed mainly of patients with other forms of human prion disease, suggested that the pulvinar sign occurred frequently in advanced cases of vCJD.[26] These investigators reported a sensitivity of up to 86% and specificity of up to 96% in this series, but suggested that this sign might be a late feature of the disease process. Histologically confirmed cases of vCJD with minimal or absent pulvinar changes on MR neuroimaging at a mean 10·5 months during an illness of mean 15 months duration were identified in this series. Values of 81% sensitivity and 94% specificity have also been reported in a series including 27 cases of vCJD

diagnosed by tonsil biopsy.[27] As these studies suggest, the pulvinar sign is not specific for vCJD. These MRI appearances are described in sporadic CJD[28] and paraneoplastic limbic encephalitis,[29] both of which are important considerations in the differential diagnosis of patients with suspected vCJD. Pulvinar signal change on MRI is also reported in several rare conditions that might otherwise be distinguished from vCJD on clinical grounds, such as benign intracranial hypertension,[30] status epilepticus associated with cat scratch disease,[31] Alpers' disease,[32] and post infectious encephalitis.[33] As this present case further exemplifies, the absence of characteristic MR findings does not exclude a diagnosis of vCJD. In fact, this case provides important insights into the relation of the pulvinar sign to clinical progression of the disease, suggesting that it could be a late feature. Quantitative MR spectroscopy studies in a small series of patients raises the possibility that more sensitive techniques might be helpful earlier in the clinical course.[34]

Tonsillar biopsy remains the most sensitive and specific diagnostic procedure for vCJD.[2,3,14,25,33] Tonsillar $PrP^{Sc}$ is uniformly present in clinically affected cases of vCJD but not in other forms of human prion disease, including iatrogenic CJD associated with use of human cadaveric derived pituitary hormones, arguing that this distinctive pathogenesis relates to effect of prion strain rather than to a peripheral route of infection.[2,3,35] As infection of lymphoreticular tissues is thought to precede neuroinvasion, and indeed has been detected in archived surgical samples removed before development of vCJD,[36,37] it is likely to allow firm diagnosis at the early clinical stage or indeed pre-clinically.[7] The case described here confirms that tonsillar infection occurs in secondary vCJD prion infection, at least in *PRNP* 129MM individuals. This finding is also of relevance to interpretation of results in due course from analysis of the National Anonymous Tonsil Archive, which aims to screen 100 000 tonsils for disease-associated PrP to estimate prevalence of vCJD prion infection in the UK population.[38] Given the advent of experimental therapeutics for prion disease, including the MRC PRION-1 trial[7,10] tissue diagnosis might be considered for asymptomatic individuals from high-risk groups so that early access to therapeutic trials can be offered, ideally before neuroinvasion, at a stage when infection may be more accessible or responsive to therapeutic intervention. Until further data are obtained on lymphoreticular pathogenesis in patients with the *PRNP* 129 VV and MV genotypes it would be prudent to consider biopsy of lymph nodes as well as tonsil in such patients. Since the successful interruption of disease progression in peripherally infected laboratory mice with monoclonal antibodies against prion protein, the future use of humanised versions of these antibodies in secondary prophylaxis might also be possible in individuals known to be at high risk of developing clinical forms of human prion disease.[39] All individuals found to have received potentially infected blood are now notified of their risk status, inevitably causing distress and uncertainty. Counselling in this situation, including discussion of public-health risks and management options, has parallels with counselling for people at risk of inherited forms of prion disease. Individuals will differ in their wishes for further investigation and early diagnosis, but all should be given the opportunity of immediate access to specialist advice, assessment, and long-term support.

#### Contributors

S J Wroe, S Pal, D Siddique, H Hyare, and R Macfarlane were responsible for the clinical case report and MRI findings, P Hewitt collected and provided blood transfusion data. J D F Wadsworth and S Joiner did biochemical analyses. S Brandner and J Linehan did neuropathological analyses. All authors contributed to drafting the manuscript and approved the final version. J Collinge directed the study, had full access to all the data, and had final responsibility for the decision to submit for publication.

#### Conflict of interest statement

JC is a director and JC and JDFW are shareholders and consultants of D-Gen Limited, an academic spin-out company working in prion disease diagnosis, decontamination, and therapeutics. D-Gen markets one of the routine antibodies (ICSM 35) used in this study. The other authors declare that they have no conflict of interest.

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| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | LANCET 2006; 368: 2226-2230 | 公表国<br>アメリカ |
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**研究報告の概要**

TSEは輸血によって感染する。献血血液からの原因病原体を選択的に吸着するのが、このリスクを処理する最も良い方法の1つであるかもしれない。我々が行った研究では、ヒト赤血球製剤にスパイクした脳由来の感染性物質を約4 log ID50減らすことのできるアフィニティ樹脂L13と製造スケールで製造された同等能力のL13Aとについて、血液中に存在する内因性TSE感染性物質の除去能力を評価した。スクレイピーに感染させたハムスターの全血500mLを使用し、白血球除去によって感染性の72%が除去された。白血球除去した全血をそれぞれのアフィニティ樹脂1mL当り50mLを処理した。白血球除去した全血を接種した99匹の内、15匹が感染したが、各々の樹脂を通過して得られた最終産物を接種された96匹又は100匹はいずれも、540日後に発症しなかった。このバイオアッセイの検出限界は0.2感染用量/mlであった。白血球除去した方の感染性の減少は全体で1.22 log ID以上であった。
この結果は白血球除去した全血から、アフィニティ樹脂によって内因性TSE感染性物質が除去されることを示していた。この同じ樹脂は血液成分の存在下で、変異型、孤発型及び家族性のCJDからの正常及び異常プリオン蛋白を吸着する。

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wfの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| スクレイピーに感染させたハムスターの白血球除去後の全血をアフィニティ樹脂で処理することにより、その感染性を除去したとの報告である。<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

Articles

# Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins

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See Comment page 2190

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## Summary

**Background** Transmissible spongiform encephalopathies (TSE) can be contracted through blood transfusion. Selective adsorption of the causative agent from donated blood might be one of the best ways of managing this risk. In our study, affinity resin L13, which reduces brain-derived infectivity spiked into human red blood cell concentrate by around 4 $\log_{10}ID_{50}$, and its equivalent, L13A, produced on a manufacturing scale, were assessed for their ability to remove TSE infectivity endogenously present in blood.

**Methods** 500 mL of scrapie-infected hamster whole blood was leucoreduced at full scale before passage through the affinity resins. Infectivity of whole blood, leucoreduced whole blood (challenge), and the recovered blood from each flow-through was measured by limiting dilution titration.

**Findings** Leucoreduction removed 72% of input infectivity. 15 of 99 animals were infected by the challenge, whereas none of the 96 or 100 animals inoculated with the final flow-throughs from either resin developed the disease after 540 days. The limit of detection of the bioassay was 0·2 infectious doses per mL. The overall reduction of the challenge infectivity was more than 1·22 $\log_{10}ID$. The results showed removal of endogenous TSE infectivity from leucoreduced whole blood by affinity ligands. The same resins adsorb normal and abnormal prion protein from human infections with variant, sporadic, and familial Creutzfeldt-Jakob disease, in the presence of blood components.

**Interpretation** TSE affinity ligands, when incorporated into appropriate devices, can be used to mitigate the risks from TSE-infected blood, blood products, and other materials exposed to TSE infectivity.

## Introduction

Experimental and natural transmissible spongiform encephalopathy (TSE) diseases can be contracted via blood transfusion (Rohwer Laboratory, ongoing research).[1–6] There have been three known transfusion transmissions of variant Creutzfeldt-Jakob disease (vCJD) in humans from a small group of known pre-symptomatic donors, indicating high infection efficiency. A survey of surgically removed tissues estimated the number of individuals infected with vCJD in the UK population to be around 4000, assuming 100% ascertainment. This prevalence is greater than that estimated from the 199 symptomatic cases recognised to date and forewarns that blood donations from presymptomatic individuals present a serious threat of human to human transmission.[3–5,7,8] To reduce this risk, deferral of potentially exposed donors has been implemented in many countries.[9] However, this option is not available for highly exposed populations, such as in the UK. Although testing individual donations is desirable, the technical challenges for such a test remain formidable.[10,11] Most of these obstacles can be circumvented by appropriately designed and validated removal strategies, which in the end might also provide greater protection from these low-titre, long incubating agents than is even theoretically possible by diagnostic strategies.

To obtain highly specific ligands that bind the prion protein (PrP), we screened millions of ligands from combinatorial libraries[12,13] and other materials for selective binding to both normal ($PrP^{c}$) and abnormal ($PrP^{res}$) prion proteins from humans, hamsters, and other species[14,15] in the presence of blood and blood components. Ligands and their resin supports were further selected for lack of negative effects on red blood cells and plasma proteins. Strong binding to human PrP was confirmed using tissue from people with sporadic CJD (sCJD) and vCJD, as well as mouse-adapted familial CJD (fCJD).

The best resins were further assessed in column format for binding normal human $PrP^{c}$ prepared from human platelets, abnormal human $PrP^{res}$ from sCJD brain homogenates spiked into human red blood cell concentrates, and PrP from normal and scrapie-infected hamster brain homogenates spiked into human red blood cells. From this analysis seven resins were chosen for infectivity studies. Each was challenged in column format with a unit of human red blood cells spiked with hamster brain homogenate infected with 263K scrapie. Three resins reduced the spiked infectivity by around 4 $\log_{10}ID_{50}$. More than $10^{9}$ infectious doses of spiked infectivity were bound, which is at least six orders of magnitude greater than the infectivity expected for a unit of leucoreduced red blood cell concentrate from an

infected donor.[16] Nevertheless, a small fraction (1:10000) of the brain infectivity could not be removed,[16] raising the question of whether the endogenous TSE infectivity present in blood might be in this capture-resistant form. To address this question and to satisfy other reservations about the relevance of brain-derived infectivity spikes for blood studies, the resin that proved most effective in the spiking study, L13, was tested for its ability to remove TSE infectivity from leucoreduced, scrapie-infected blood.

## Methods

We obtained blood from around 130 symptomatic hamsters infected by the intracranial route with a low dose of the 263K strain of scrapie as previously described.[17] The blood was pooled to produce a human-size unit (505 mL) and kept at ambient temperature throughout the study. A sample (15 mL) of whole blood was removed for titration. The remaining blood was leucoreduced with a commercial Pall WBF2 filter (Pall Corporation, Port Washington, NY, USA) following the manufacturer's instructions. The reduction in hamster white blood cell concentration was confirmed to be around 3 $\log_{10}$ (to 25 cells per µL).[17] A 15 mL sample of leucoreduced blood (challenge) was removed for titration.

Two ligands immobilised on the same type of chromatographic resin support were tested: L13, previously described,[16] and L13A, a version of the same resin produced at manufacturing scale. Roughly 50 mL of the leucoreduced hamster infected blood was applied to 1 mL of each resin packed in disposable columns (PIKSI kit, ProMetic Biosciences, Cambridge, UK). The column flow rate was controlled with a peristaltic pump (Rainin Instrument, Woburn, MA, USA) at 0·5 mL/min. Each resin was divided between two chromatography columns, which were challenged in series by a common pool of leucoreduced, scrapie-infected hamster whole blood. For each resin, the flow-through from column 1 (flow-through 1) was used to challenge column 2 (flow-through 2). The column-in-series strategy was used, as in the brain spike study,[16] with two objectives: to confirm the volume of resin needed to clear the infectivity and to assess the intrinsic susceptibility of the infectivity to removal. When infectivity is present in flow-through 1 but not in flow-through 2, all of the infectivity can be adsorbed but two column volumes are needed. If the infectivity that escaped column 1 also escaped column 2, we could conclude that there is a form of infectivity that is not captured by the resin.

Samples of the blood before and after leucoreduction and samples of the flow-throughs 1 and 2 of both resins were titred. For each resin, the first flow-through was collected in a single fraction, mixed thoroughly, and 12 mL removed for titration. The remainder was applied to an identical second column and the flow-through 2 again collected in a single fraction, mixed thoroughly, and stored for titration.

Titrations were by the limiting dilution method.[17] This method is a precise and sensitive way to measure TSE infectivity in low-titre samples such as blood. About 10 mL of each sample was ultrasonicated at 40% full power in four 15 s pulses on ice (Sonics and Material, Vibra cell, VCX 750 and stepped microprobe, Newtown, CT, USA), before inoculation of weanling golden Syrian hamsters with 50 µL per animal. The titre was calculated as previously described.[17]

Whole blood was inoculated into about 50 animals, whereas all other samples were inoculated into roughly 100 animals. Animals that died at the time of inoculation or before 21 days' incubation were removed from the total count, since the short incubation time was not consistent with scrapie infections. Altogether, this loss represented about 10% of the total animals inoculated and is due to toxic consequences of the intracranial inoculation of blood. Animals were isolated when they showed the first symptoms of scrapie and were subsequently observed daily. The brains of all animals that contracted scrapie or died intercurrently or were terminated for unrelated causes were collected. At 544–45 days after inoculation, all animals still alive were killed and the brain of each animal was removed.

Every brain was tested for the presence of the proteinase K-resistant protein, $PrP^{res}$. Individual brains were homogenised in phosphate buffered saline pH 7·2 to 10% weight per volume with a MediFASTH homogeniser (Consul AR, Villeneuve, Switzerland). The assay was done according to a previously described protocol[16] with the following modifications: in one 100 µL sample, proteinase K was added at 0·1 mg/mL final concentration, and in another 100 µL sample the enzyme was replaced with phenylmethylsulphonyl fluoride at 1·7 mg/mL final concentration. Both reaction mixtures contained 2% sodium dodecyl sulphate (SDS). The reaction mixtures were incubated at 37°C for 20 min followed by 100°C for 10 min to stop the digestion and denature the $PrP^{res}$. The samples were diluted 100-fold in DELFIA (Dissociation Enhanced Lanthanide Fluorescence Immuno Assay)[18] assay buffer (PerkinElmer, Wellesley, MA, USA) before testing for the presence of $PrP^{res}$ with purified 3F4 (Signet, Dedham, MA, USA) as the capture antibody, and purified 7D9 (Signet) labelled with Europium as the detection antibody. Antibody labelling was done according to the manufacturer's instructions (PerkinElmer) and the molar ratio of Europium:7D9 antibody was 7·4:1. Animals that died intercurrently and all animals with inconclusive DELFIA results were assayed for the presence of $PrP^{res}$ by western blot with 3F4 monoclonal antibody.[16]

### Role of the funding source

This work was funded by Pathogen Removal and Diagnostic Technology (PRDT) Inc and by MacoPharma, a worldwide manufacturer of in-line blood filters and blood collection sets. PRDT is a joint venture of the American National Red Cross and ProMetic BioSciences.

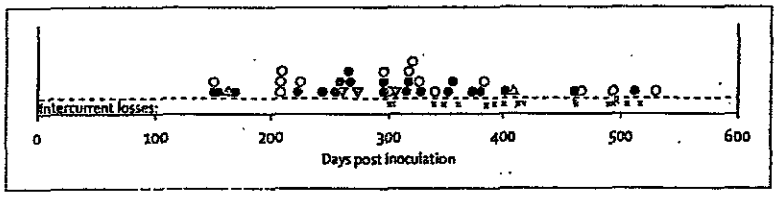


*Figure:* Distribution of incubation times for all infections in the study
598 animals were inoculated. Animals that died from complications during inoculation or before 21 days following inoculation were excluded from the calculations (around 10%). Only animals that became infected with scrapie or died intercurrently of unrelated causes are plotted (58 of 540 that survived past 21 days). 89% of the 540 experimental animals survived without infection to the end of the experiment at 540 days. Whole blood before leucoreduction (black circles), leucoreduced whole blood (white circles), L13 flow-through 1 (down triangles) and L13A flow-through 1 (up triangles), intercurrent losses unrelated to scrapie (x).

PRDT was involved at all levels of this study: in the design and execution of the study, the interpretation of data, the decision to submit the paper for publication, and in the writing of the manuscript. MacoPharma supported this study financially. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

## Results

The incubation times of each infection in this study are shown in the figure. The total volume and number of animals inoculated for each sample, and the number of animals that contracted scrapie, are reported in the table along with the calculated titres and standard deviations of each sample. The brains of all animals in the study were analysed for $PrP^{res}$ to confirm the clinical scoring and to discover incubating infections at the time of termination. Only one animal (challenge group), which died of intercurrent causes at 530 days after inoculation, was detected by western blot to be incubating scrapie. All other animals that were clinically normal were negative for $PrP^{res}$ by DELFIA or western blot, while all symptomatic animals had $PrP^{res}$ in their brains.

In this study, leucoreduction removed 72% of the infectivity present in whole blood compared with an earlier study, in which only 42% infectivity was removed.[9] Two of 99 animals inoculated with flow-through 1 of L13 resin were infected, as were three of 99 animals inoculated with flow-through 1 of L13A resin. This result corresponded to a decrease in titre from 3·3 (SD 0·8) ID/mL (leucoreduced whole blood) to 0·4 (0·3) and 0·6 (0·4) ID/mL for L13 and L13A, respectively (table). By contrast, none of the 100 and 96 animals inoculated with flow-through 2 of either resin developed the disease during 540 days of incubation. Thus, there was complete removal of the 16 ID present in the 4·95 mL of challenge inoculated. For the entire processed volume (table), infectivity was reduced to below the limit of detection of the assay, which is one infection in 5 mL inoculated or 0·2 (0·2) ID/mL.

The $\log_{10}$ of infectivity reduction calculated for each filtration step individually and cumulatively are reported in the right-hand columns of the table. The first passage reduced the infectivity in the challenge by 0·91 $\log_{10}$ID and 0·73 $\log_{10}$ID for L13 and L13A, respectively. The infectivity that passed the first column was captured by the second column. Residual infectivity, if any, was below the limit of detection. The cumulative infectivity reduction for the two columns was greater than 1·22 $\log_{10}$ID.

## Discussion

Blood-associated TSE infectivity presents unique challenges to risk management absent from other blood-borne pathogens. The low concentration of infectivity, although difficult to detect, has nevertheless been sufficient for efficient transfusion transmission of these fatal diseases. People with certain genotypes could harbour the infection in a transmissible but undetectable form for decades. The form that the infectivity takes in

| | Volume processed (mL) | Total volume inoculated (mL) | Total animals assayed | Total animals infected | Calculated titre | | Total measured infectivity | | | Reduction factor* | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Titre† (ID/mL) | SD‡ (ID/mL) | Challenge (ID) | Unbound (ID) | Removed (ID) | Stepwise ($\log_{10}$ID) | Cumulative ($\log_{10}$ID) |
| Whole blood | 490 | 2·35 | 47 | 21§ | 11·8 | 2·2 | | | | | |
| Leucoreduced blood | 490 | 4·95 | 99 | 15 | 3·3 | 0·8 | 59 | 16 | 42 | 0·56 | |
| Challenge | | 4·95 | 99 | 15 | 3·3 | 0·8 | | | | | |
| L13 flow-through 1 | 51 | 4·95 | 99 | 2 | 0·4 | 0·3 | 16 | 2 | 14 | 0·91 | |
| L13 flow-through 2 | 40 | 4·80 | 96 | 0 | <0·2¶ | 0·2 | 2 | 0 | >2 | 0·31 | >1·22 |
| L13A flow-through 1 | 50 | 4·95 | 99 | 3 | 0·6 | 0·4 | 16 | 3 | 13 | 0·73 | |
| L13A flow-through 2 | 38 | 5·00 | 100 | 0 | <0·2¶ | 0·2 | 3 | 0 | >3 | >0·49 | >1·22 |

*Reduction factors are calculated as $\log_{10}$(challenge titre)–$\log_{10}$titre remaining after reduction step or when there is no remaining infectivity, the titre at the limit of detection). †$Titre_{max}$=[–ln [(total animals assayed–total animal infected)/(total animals assayed)]]×[(1 ID/50uL)×(1000 uL/mL)]. ‡$SD_{max}$=[($titre_{max}$)/(total volume $inoculated_{max}$)]$^{1/2}$. §Less than half as much volume was inoculated for this sample as the others. ¶Limit of detection in a limiting dilution titration is a single infection in the cohort inoculated. For a cohort, c, the probability of infection by a single inoculation at the limit of detection titre is 1/c. Probability of no infections, P (0), at that titre is (1–1/c). Probability of no infections in an entire cohort inoculated at the limit of detection titre is $P_c(0)=[P(0)]^c$. Probability of observing at least one infection at a given P (0) is $P_c(\geq 1)=1-P_c(0)$. For no infections in 100 inoculations of 50 uL, there is a minimum probability of 0·63 that the concentration of infectivity is ≤0·20 ID/mL, the limit of detection, and a minimum probability of 0·90 that it is ≤0·46 ID/mL. Since the actual titre is unknown and could be far less than the limit of detection, these values represent upper limits on the actual titre.

*Table:* Distribution of infectivity in whole blood, challenge, and resin flow-through

blood is unknown but might be different from that associated with brain. No pathogen-specific antibodies have been identified for diagnosis, and infection-associated forms of the prion protein constitute only a minuscule proportion of the total prion protein present in blood, thereby presenting a formidable challenge to detection.

Selective adsorption of TSE infectivity from donated blood might be one of the best ways of managing this risk. Ligands that bind all forms of the prion protein with high affinity can still remove trace amounts of infection-associated forms in the presence of much higher concentrations of normal forms. Binding will take place even at the earliest times in the infection when the concentration of infectivity is below the limit of detection of diagnostic assays. Finally, if every donation is treated, the protection might be more comprehensive than from a diagnostic test.

In this study, powerful affinity ligands, selected from libraries of millions of compounds and materials, were tested for their ability to remove the low amounts of TSE infectivity that are naturally present in the blood of infected individuals. After increasingly stringent screening, resin L13 was selected for assessment with endogenous blood infectivity. Optimisation to facilitate manufacturing of this resin produced L13A. The two resins are identical in terms of ligand chemistry and matrix, and are functionally equivalent.

Universal leucoreduction has been implemented in several countries, including the UK, in part to remove white cell-associated TSE infectivity. However, we previously showed that leucoreduction removed less than half of blood-borne TSE infectivity.[9] Other evidence suggests that the remaining infectivity is in plasma.[9] To further manage TSE risk, the UK no longer fractionates its domestic plasma. That leaves the bulk of the residual risk with red blood cell concentrate, which is by far the most frequently used blood component, but one for which there is no alternative to domestic sourcing. Red blood cells are for this reason in most urgent need of risk mitigation and have been the first objective for TSE removal. However, because of the low and variable amounts of plasma in leucoreduced red blood cell concentrates, the infectivity in a red blood cell preparation from hamsters might be insufficient to show a meaningful level of removal. Instead, leucoreduced whole blood from hamsters infected with 263K scrapie was used to challenge resins L13 and L13A. Hamster 263K scrapie has consistently produced blood infectivity titers of 10 (SD 2) ID/mL during symptomatic disease (Rohwer Laboratory, unpublished).[10] Since leucoreduced whole blood retains its full complement of plasma and red blood cells, and since removal was to the limit of detection, the same resins would probably also remove endogenous infectivity from plasma, plasma derivatives, and red blood cell concentrates.

The main objectives of this study were to confirm that the volume of resin needed to capture all measurable blood-borne infectivity, as deduced from earlier experiments, was correct, and to make sure that the infectivity in blood was in a form that could be adsorbed by the resins. In an earlier study, one part per 10 000 of the infectivity in a brain spike was not removable by any resin tested.[6] In this study, resins in the proportion of 2 mL to 50 mL leucoreduced blood removed the endogenous, blood-associated infectivity to below the limit of detection (0·2 ID/mL). With a challenge of 3·3 ID/mL of leucoreduced whole blood, we were able to show a greater than 1·22 $\log_{10}$ID reduction. All of the infectivity in the roughly 5 mL volume inoculated was removed before the final flow-through of both resins. Because there were no infections, we can calculate from the Poisson distribution that there is a 63% minimum probability that the concentration of infectivity is 0·20 ID/mL or less, ie, the limit of detection, and a 90% minimum probability that it is 0·46 ID/mL or less. Since the actual titre is unknown and could be far less than the limit of detection, these values represent upper limits on the actual titre. In this experiment, the endogenous infectivity in a full unit of leucoreduced whole blood (450 mL) would be 1485 ID. Passage through the resin would reduce the infectivity to, at maximum, 90 remaining infectious doses. This corresponds to, at minimum, 1395 infectious doses removed.

The reduction efficiency of the resin differed for endogenous blood-associated infectivity and spiked brain-derived infectivity. Between 1 and 2 mL of resin were needed to capture the 165 ID from 50 mL of leucoreduced hamster blood versus 20–30 mL of the same resin to capture several million infectious doses from brain spiked in a 330 mL unit of human red blood cell concentrate.[6] Important differences between the studies include the source of infectivity—blood versus brain; the protein environment—human red blood cell concentrates versus hamster whole blood; and the flow rate—gravity versus pump. Both approaches provided valuable information and our experience emphasises the value of experiments that use both methods.

The figure shows that symptomatic disease in limiting dilution titration assays occurs randomly from 120 days to more than 500 days, and only rarely after 500 days incubation.[9] Titre cannot be deduced from incubation times, and premature termination would lead to overestimates of removal capabilities and underestimates of titre. However, extending the endpoint beyond 540 days is not beneficial, since new infections are rare, whereas age-related mortality increases greatly (Rohwer Laboratory, unpublished).

In a previous study, leucoreduction removed around 42% of the infectivity in hamster whole blood[9] compared with 72% in this study following the same protocol. Both filtrations used human-sized units of whole hamster blood and both filters worked to specification for removal of white blood cells. The differences in infectivity removal could indicate natural variability between blood pools, filters, stability of white blood cell associations, or subtle

differences in execution. Differences we are aware of include: the collection interval—7 h in the first experiment and 4 h in the second; and the filtration time—about 20 min in the first filtration and around 11 min in the second.[P] Neither filtration removed sufficient infectivity to eliminate the risk. Thus, a strong TSE blood safety programme cannot rely only on leucoreduction. We note also that the 30% difference between these two results could not have been detected by any other means than limiting dilution titration.

Resins L13 and L13A, which were indistinguishable in these studies, adsorb endogenous TSE infectivity from leucoreduced whole blood. Both resins capture $PrP^{res}$ from infected human and hamster brain diluted into human whole blood and plasma, and $PrP^{c}$ from human whole blood and plasma (data not shown). These protein-based data from people and hamsters, together with this study showing removal of endogenous infectivity from hamster blood, makes adsorption of TSE infectivity from human blood, plasma, and red blood cell concentrates by these resins highly probable. Resin L13A has been incorporated into a filter device format, P-CAPT (MacoPharma, Lille, France), for reduction of human endogenous TSE infectivity in leucoreduced red blood cell concentrates.

**Contributors**

The overall design and execution of the experiment, including management of the logistics and all the infectivity work, was by L Gregori, B C Lambert, and R G Rohwer with the assistance of the staff of the Molecular Neurovirology Laboratory. P V Gurgel, R G Carbonell, S J Burton, and D J Hammond provided expertise on resins, combinatorial peptide and polymer libraries, and chromatography. J T Lathrop and D J Hammond supervised the blood studies. P Edwardson coordinated the efforts of several laboratories to the final goal of this study.

**Conflict of interest statement**

R G Rohwer, R G Carbonell, and D J Hammond are cofounders and part owners of Pathogen Removal and Diagnostics Technologies, which sponsored this study together with MacoPharma. S J Burton, P V Gurgel, and P Edwardson are employees of ProMetic BioSciences, a joint venture partner in the development of the removal resin. D J Hammond and J T Lathrop are employees of the American National Red Cross, also a joint venture partner in the development of the resin. L Gregori and B C Lambert receive contract support from Pathogen Removal and Diagnostics Technologies for these and other studies on TSE removal resins.

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**研究報告の概要** 709

○乳汁中のプリオンタンパク質
【背景】プリオンは、中枢神経系に蓄積した後に伝達性海綿状脳症(TSE)を引き起こすことが知られている。プリオンは体液中にも存在し、輸血によりプリオンが感染する可能性があるというエビデンスが増えつつある。体液中のタンパク質性病原体濃度の低さと当該病原体の潜伏期間の長さが、疫学的分析並びに伝播の推定(すなわちヒトへの感染リスクの推定)を困難にしている。この感染性病原体をモニターするための感度の高い方法はなく、これは特に食品医薬品業界では問題である。
【方法/主な知見】プリオンタンパク質に特異的かつ高親和性で結合する吸着マトリックス、Alicon PrioTrap®を開発した。これを用いてヒト、ウシ、ヒツジ、ヤギの乳汁中のプリオンタンパク質(PrPC、プリオンPrPScの前駆体)が特定できた。PrPCの絶対量には種間差があった(ヒツジの乳中でμg/Lレンジ～ng/Lレンジ)。PrPCは、均質化し低温度殺菌した市販ミルクからも認められ、超高温処理を施しても内因性PrPCの濃度はわずかにしか減少しなかった。
【結論/有意性】乳腺炎に罹患したスクレイピー感染ヒツジの乳腺中のプリオン複製の証拠を示す最近の研究を考えれば、乳汁中にPrPCが出現することは、TSE感染動物の乳汁がPrPScの感染源となる可能性を示唆する。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| ヒト、ウシ、ヒツジ、ヤギの乳汁及び均質化し低温度殺菌した市販ミルクからPrPCが検出されたとの報告である。 | 今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。 |

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PLoS one

# Prion Protein in Milk

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***Background.*** Prions are known to cause transmissible spongiform encephalopathies (TSE) after accumulation in the central nervous system. There is increasing evidence that prions are also present in body fluids and that prion infection by blood transmission is possible. The low concentration of the proteinaceous agent in body fluids and its long incubation time complicate epidemiologic analysis and estimation of spreading and thus the risk of human infection. This situation is particularly unsatisfactory for food and pharmaceutical industries, given the lack of sensitive tools for monitoring the infectious agent. ***Methodology/Principal Findings.*** We have developed an adsorption matrix, Alicon PrioTrap®, which binds with high affinity and specificity to prion proteins. Thus we were able to identify prion protein ($PrP^C$)–the precursor of prions ($PrP^{Sc}$)–in milk from humans, cows, sheep, and goats. The absolute amount of $PrP^C$ differs between the species (from µg/l range in sheep to ng/l range in human milk). $PrP^C$ is also found in homogenised and pasteurised off-the-shelf milk, and even ultrahigh temperature treatment only partially diminishes endogenous $PrP^C$ concentration. ***Conclusions/Significance.*** In view of a recent study showing evidence of prion replication occurring in the mammary gland of scrapie infected sheep suffering from mastitis, the appearance of $PrP^C$ in milk implies the possibility that milk of TSE-infected animals serves as source for $PrP^{Sc}$.

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## INTRODUCTION

Prion protein was detected in attempts to identify the infective agent of TSE [1,2]. The finding that prion protein is present in normal and TSE-infected brain at similar levels [3,4] suggests that the "cellular" prion protein ($PrP^C$) constitutes a precursor of the "scrapie" prion protein ($PrP^{Sc}$) causing TSE such as bovine spongiform encephalopathy (BSE) in cattle or Creutzfeldt-Jakob disease (CJD) in humans. There is convincing evidence that the transition from precursor protein to infectious prion is due to a major conformational transition [5].

Prion protein is highly conserved among mammals [6]. It is primarily synthesized in cells of the central nervous system [7], but is also abundantly expressed in several peripheral tissues [8,9]. An amino-terminal signal sequence targets prion protein to the endoplasmatic reticulum, where it transits the Golgi and ultimately reaches the external surface of the cell membrane [10]. There it is attached to a carboxy-terminal glycosyl phosphatidylinositol anchor [11]. The mature bovine protein of 217 amino acids contains two consensus acceptor sites for addition of N-linked polysaccharides [12].

Prion proteins ($PrP^C$ and $PrP^{Sc}$) have been detected in the cellular fraction of blood [13–17], but so far not in milk [18–21]. Considering that milk and milk products represent a major component of human nutrition it seems of particular importance to analyze milk for the presence of prion proteins. A first step in this direction is to determine the amount of $PrP^C$ in milk of healthy animals. If milk contains a significant amount of $PrP^C$, this could indicate that $PrP^{Sc}$ might be present in so far undetectable amounts in milk of TSE infected animals. However, the high concentration of total protein (about 40 mg/ml) and the high amount of lipids (about 35 mg/ml) in the milk make prion protein analysis by common biochemical methods demanding. We have therefore developed an adsorption matrix, Alicon PrioTrap®, which binds with high affinity and specificity to prion proteins $PrP^C$ and $PrP^{Sc}$. The exceptional binding properties of Alicon PrioTrap® result from hydrophilic and hydrophobic surface clusters that recognize different prion protein epitopes, allowing quantitative enrichment of extreme low quantity of prion proteins in body fluids and in biological tissues.

## RESULTS

The detection of native $PrP^C$ after enrichment from 10 ml milk from cow, sheep, goat, and human using Alicon PrioTrap® is shown in figure 1. In cow milk three $PrP^C$ isoforms are observed with an apparent molecular mass of about 34 kD, 30 kD, and 27 kD corresponding to diglycosylated, monoglycosylated, and unglycosylated $PrP^C$, respectively. In some preparations monoglycosylated $PrP^C$ appears as a double band, indicating that the two glycosylation sites may be linked to different carbohydrates. The apparent molecular mass of unglycosylated $PrP^C$ is slightly higher when compared to a recombinant bovine PrP(25–241) standard at 26 kD, indicating that native $PrP^C$ in milk contains a glycosyl phosphatidylinositol anchor [11]. About the same distribution of $PrP^C$ isoforms is observed for sheep, goat, and human milk, although the total amount of native $PrP^C$ significantly differs between the species. The relative ratio of sheep/cow/goat/human $PrP^C$ is estimated at 100/20/4/1. From experiments performed on sequential incubations with Alicon PrioTrap® the total concentration of $PrP^C$ in fresh cow milk can be estimated to be about 200 pg/ml. Taking into account the

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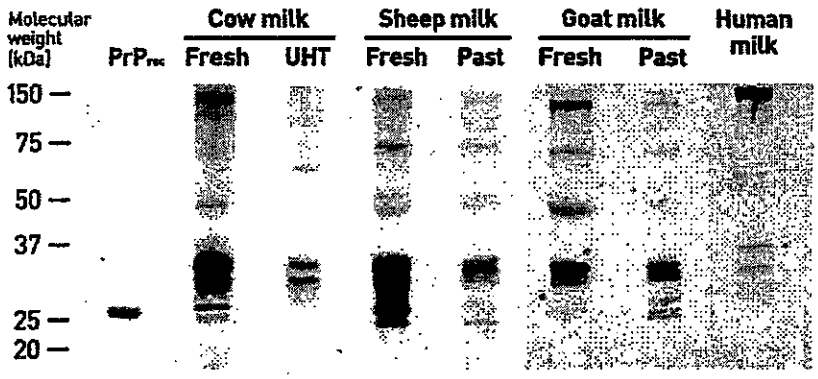


**Figure 1. Detection of native PrP$^C$ in milk of human and animals.** A volume of 10 ml fresh, ultra-high temperature treated (UHT) and pasteurised (Past) milk were enriched for PrP$^C$ using Alicon PrioTrap® technology. Concentrated PrP$^C$ was analyzed by Western Blotting using PrP—mab 8B4 (Alicon AG). The molecular weight markers are indicated. Recombinant bovine PrP(25—241) (Alicon AG) was used as a standard.
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relative ratios of PrP$^C$ in milk of different species, fresh sheep milk and goat milk contain about 1 ng/ml and 40 pg/ml PrP, respectively. Human breast milk contains less than 10 pg/ml PrP$^C$. The concentration of PrP$^C$ in Swiss off-the-shelf milk is reduced when compared to fresh milk, but prion protein can clearly be detected (Figure 1). About the same concentration of PrP$^C$ was measured for organic farm milk and non-organic farm milk as well as for pasteurized and ultra-high temperature (UHT) treated milk (data not shown).

To confirm specificity of immunochemical detection of PrP$^C$ in milk, we compared different anti-PrP monoclonal antibodies, which are directed against non-overlapping epitopes (Figure 2): PrP—mab 8B4 binds to residues 37—44 of mouse PrP [22]; mAB 6H4 targets residues 144—152 [23]; and PrP—mab 8H4 binds to residues 175—185 [24]. The three antibodies recognize the same proteins and thus confirm the presence of PrP$^C$ in milk. In control experiments, with non-PrP antibodies, *e.g.*, anti-Tau protein monoclonal antibody (Chemicon International) (Figure 2) and anti-Aβ monoclonal antibody (Calbiochem, Germany; data not shown), none of the PrP$^C$ isoforms was detected, thus confirming binding specificity of the anti-PrP monoclonal antibodies. An interesting observation with regard to antibody 8B4 is its "clear" detection profile when compared to 6H4 and 8H4 antibodies. This can be rationalized by 8B4 not recognizing a variety of carboxy-terminal fragments of milk PrP$^C$, which appear as smear in the Western Blot.

We further compared the glycoforms of native prion protein in cow milk with those of bovine brain, a tissue where prion protein expression is well characterized. The glycoforms were identified by digestion with PNGase (Figure 3), an enzyme that cuts off oligosaccharides from N-linked glycoproteins, *e.g.*, the two N-linked sugars of PrP$^C$ [12]. After partial cleavage with PNGase the upper PrP-isoform in the Western Blot representing diglycolysated PrP$^C$ (34 kD) disappears in favour of monoglycosylated (30 kD) and nonglycosylated PrP$^C$ (27 kD). In parallel, there seems to be a small shift from the higher molecular weight monoglycosylated form to the lower molecular weight form. A slight downshift of the monoglycosylated PrP$^C$ is also observed for brain homogenate after PNGase treatment (Figure 3). The diglycosylated PrP$^C$ isoforms differ slightly in molecular mass, indicating that carbohydrate structure of PrP$^C$ in milk and brain may not be identical. More stringent reaction conditions result in complete truncation of carbohydrates from PrP$^C$. Most importantly, the apparent molecular masses of nonglycosylated PrP$^C$ in milk exactly matches with that of the corresponding PrP$^C$ in brain homogenate.

Alicon PrioTrap® can also be applied for elimination of prion protein from milk. As shown in Figure 4, after the first treatment of 10 ml milk with Alicon PrioTrap® more than 95% of endogenous PrP$^C$ was already removed, and after the second treatment PrP$^C$ was completely eliminated. However, the overall protein concen-

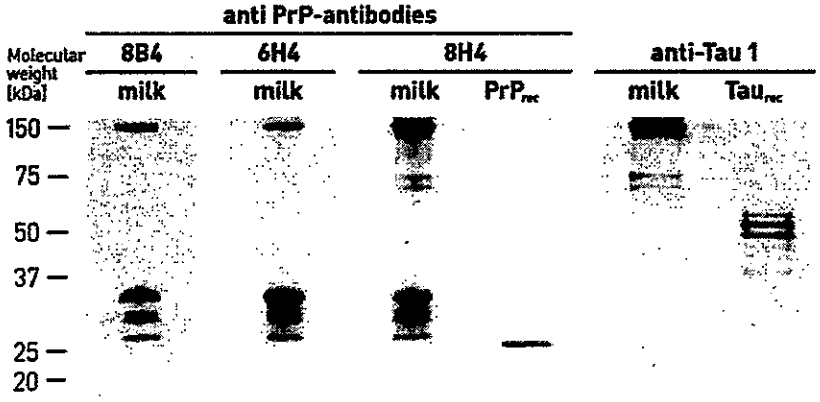


**Figure 2. Specific binding of anti-PrP monoclonal antibodies to milk PrP$^C$.** Various anti-PrP monoclonal antibodies were used for detection of PrP$^C$ in fresh cow milk. A Tau-1 protein-specific monoclonal antibody was used as a negative control. Recombinant bovine PrP(25—241) (Alicon AG) and recombinant Tau-1 protein (Chemicon International) were used as a standard.
doi:10.1371/journal.pone.0000071.g002

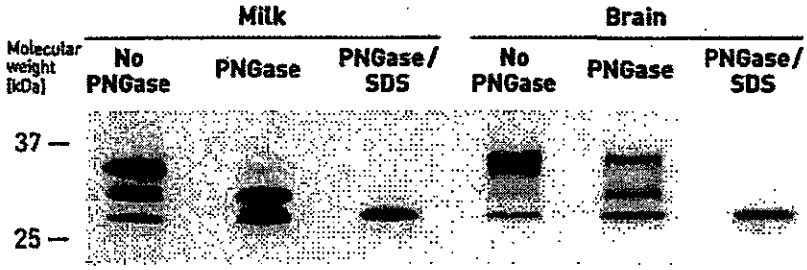


**Figure 3. Glycoform patterns of milk and brain $PrP^C$.** Alicon PrioTrap®-enriched $PrP^C$ from cow milk and brain homogenate was treated with PNGase or PNGase/SDS before Western Blotting using PrP–mab 8B4.
doi:10.1371/journal.pone.0000071.g003

tration (measured by bicinchoninic acid assay, Pierce) was constant with about 40 mg/ml before and after $PrP^C$ elimination. The protein composition of milk as analyzed by SDS PAGE (Figure 4) was not affected either. Prion protein was also completely removed, when milk was spiked with $PrP^{Sc}$ from mouse Rocky Mountain Laboratory (RML) brain homogenate (data not shown). Thus, Alicon PrioTrap® can be used for enrichment and detection of overall prion protein in milk, but also for complete removal of prions.

## DISCUSSION

Milk contributes 13% to the worldwide protein supply for humans. World milk production ranges around 500 million tons a year. Before fresh milk reaches the consumer, it is usually homogenized to reduce fat particle size in order to increase digestibility of the milk and heated. Surprisingly, pasteurisation (heating for 30 seconds to 72°C) and ultra-high temperature treatment (heating for 1–4 seconds to 135°C) only leads to a partial reduction of the amount of $PrP^C$. This supports the observation that $PrP^C$ is highly stable in milk. Thus, the heating procedures used to inactivate DNA-containing pathogens are not sufficient to eliminate endogenous prion proteins.

The presence of $PrP^C$ in blood has been documented [14,15], and is confirmed by our own unpublished observations. To produce one liter of milk, about 400 to 500 liters of blood must pass through the udder of a cow. It is thus possible that the $PrP^C$ found in milk derives from blood cells or, alternatively, has been secreted from glandular epithelial cells. Cell types that have been identified in milk from healthy cows are mainly macrophages, and other leucocytes. However, in our assay cells are completely removed by centrifugation. Therefore, the recovered $PrP^C$ is not cell associated but most likely binds to other proteins or lipids resulting in stable molecular complexes. The fact that milk contains full-length $PrP^C$, very likely comprising the glycolipid anchor, indicates that prion protein was originally cell-bound and does not represent any of the amino-terminal truncation products of $PrP^C$ known to be released from normal cells under physiological conditions [25]. The detection of such a considerable expression of cell membrane bound or derived $PrP^C$ in milk constitutes one of the key requirements for the generation of infectious prions in the udder of infected animals.

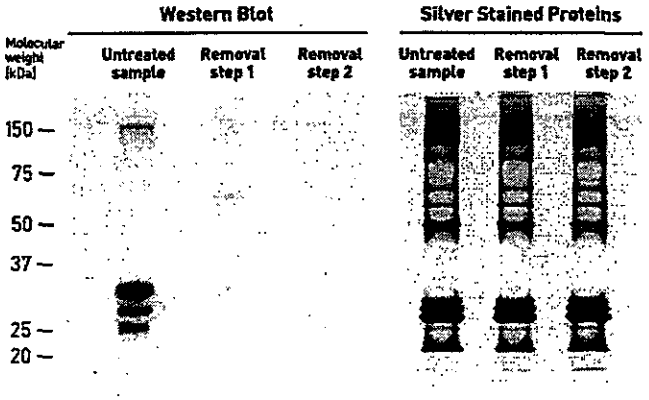


**Figure 4. Elimination of $PrP^C$ from milk.** $PrP^C$ from 10 ml fresh cow milk was detected as described in figure 1, followed by two consecutive $PrP^C$ removal steps, where the milk supernatant was incubated with Alicon PrioTrap® filtration resin for 30 min before immunochemical detection. The total amount of milk protein was analyzed by Silver Stain analysis.
doi:10.1371/journal.pone.0000071.g004

Over the last 10 years, scientific groups, risk assessment agencies, and public health organizations have debated the TSE risk for milk and milk products [26,27]. Epidemiological and bioassay data so far available have not provided evidence for milk to harbour prion infectivity and infectious prions have as yet not been detected by bioassays in the milk, colostrum or udder of clinical BSE cases in cow [18–21]. However, a recent statement of the European Food Safety Authority affirmed that based on a number of observations from research data, there are indications that infectivity in the milk from small ruminants can not be totally excluded [28]. Furthermore, the exclusion of animals with mastitis, an inflammation of the mammal gland, being able to destabilize the blood-milk barrier, is considered a measure able to reduce but not to eliminate the potential contamination risk [28]. The rational of this conclusion is confirmed by a recent study showing that in sheep naturally affected with both scrapie and lymphocyte or lymphofollicular mastitis, $PrP^{Sc}$ accumulation was present in lymphoid follicles adjacent to milk ducts [29]. At least in natural sheep scrapie, prion replication can occur following a lymphotropic virus infection in the inflamed mammary gland. This study has not detected $PrP^{Sc}$ or prion infectivity in milk itself. However, since under such inflammatory conditions, the total number of immune cells increases in milk of animals, it might be possible that infectious $PrP^{Sc}$ is also passing through and reaches the milk. In this context milk from such animals could possibly be responsible for the spread of scrapie from the ewes to their offspring in affected sheep or goat flocks. Moreover, sheep and goat milk could also constitute a TSE exposure risk for mammals (humans) consuming these products.

The former Scientific Steering committee of the European Commission and the European Food Safety Authority recommend that research should intensify on the safety of milk of small ruminants with regard to TSE risk. Limited new data are expected to be published in the near future and there is still little research initiated in this area [28]. The Alicon PrioTrap® technology opens a new avenue for studying the biochemical characteristics of prion

protein in milk and thus may contribute to offer a feasible approach to perform an appropriate study on the milk safety with regard to TSE risk.

## MATERIALS AND METHODS

### Preparation of milk samples

Fresh milk was obtained from healthy individuals and transported at 4°C. Cow UHT milk, sheep and goat pasteurised milk were obtained from the Swiss market. Each sample was prepared from 10 ml milk, centrifuged at 3000× g for 10 min to ensure complete removal of cells.

### Preparation of brain homogenate

10% (w/v) bovine brain homogenate was prepared in 100 mM Tris-HCl pH 7.5 containing 2% sodium lauryl sarcosinate. This solution was diluted in 100 mM sodium phosphate buffer pH 8 containing 0.5% NP-40 to obtain 1% (w/v) brain homogenate.

### Concentration of milk $PrP^C$

The milk supernatant was stirred for 30 min in the presence of Alicon PrioTrap®. The resin was centrifuged for 2 min at 2000× g and washed three times at RT with 10 ml washing solution containing 100 mM sodium phosphate buffer pH 8.

### Immunochemical $PrP^C$ detection

Concentrated milk and brain $PrP^C$ was denaturated in SDS sample buffer and heated at 70°C for 10 min and at 95°C for 5 min, respectively. Samples were applied to a 12% SDS polyacrylamide gel for electrophoresis and subsequently transferred to a PVDF membrane. The membrane was blocked with 2% ECL Advance™ blocking agent (Amersham) if probed with 8B4 antibody or 1% bovine serum albumin if probed with other antibodies used in this study. 8B4 antibody was incubated at a concentration of 450 ng/ml, 8H4 at 85 ng/ml, 6H4 at 285 ng/ml, and anti-Tau-1 at 50 ng/ml. Sheep anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (Amersham) was incubated at a 1/20,000 dilution. The immunoreactivity was visualized by chemiluminescence detection following the manufacture's instruction (ECL Advance Western Blot detection Kit, Amersham).

### PNGase treatment of milk and brain $PrP^C$

For PNGase treatment prion protein extracted from 10 ml cow milk or 10 µl of 1% (w/v) cow brain homogenate was incubated for 12 h at 37°C in buffer containing 100 mM sodium phosphate, 10 mM Tris-HCl, 1% NP-40, 1% MEGA-8, pH 8, and 1.5 units of N-Glycosidase F (Roche). Under more stringent cleavage conditions, proteins were denatured by heating for 10 minutes at 100°C in the presence of 0.5% SDS before treatment with 4 units of N-Glycosidase F.

### Total milk protein detection

A milk volume corresponding to 40 µg total protein (1 µl) was heated in SDS loading buffer at 70°C for 10 min. After electrophoresis on a 12% SDS polyacrylamide gel, the proteins were detected by silver staining (SilverSNAP Stain kit II, Pierce).

## ACKNOWLEDGMENTS

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### Author Contributions

Conceived and designed the experiments: ZR FN EA MU FS RZ NF AE UM SF. Performed the experiments: FN EA MU FS NF AE UM SF. Analyzed the data: ZR FN EA MU FS RZ NF AE UM SF. Contributed reagents/materials/analysis tools: AB MG SM BA GM BU MS UB. Wrote the paper: AB MG ZR FN BA GM BU RZ NF UB.

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**研究報告の概要**

○スクレイピー感染細胞およびクロイツフェルト・ヤコブ病病原体感染細胞では、細胞内に25nmウイルス様粒子が生じる
我々は、プリオンタンパク(PrP)がほとんど存在しないが、高い感染性を持つ脳において直径約25nmのウイルス様粒子を反復して認めたため、高い感染性を持つ細胞株で同様のウイルス様粒子の存在を調べた。スクレイピー22L株に感染した神経芽腫細胞およびFUクロイツフェルト・ヤコブ病病原体に感染した視床下部GT細胞は、直交状に配列した密集する25nmのウイルス様粒子が存在したが、並行対照群ではウイルス様粒子が存在しなかった。in situのこれら粒子は異常PrPアミロイドと関連性がなく、正常および異常細胞内PrPが存在するとされる部位である粗面小胞体膜やアミロイド線維を正確に認識するPrP抗体によって標識されなかった。また、ホルボールエステルはN2a+22L感染細胞の異常PrPゲルバンドの発現量を5倍以上増加させたが、ウイルス様粒子の列数やこれら細胞の感染価は増加しなかった。したがって、25nmの当該感染関連粒子はプリオンではあり得なかった。シナプス分化や神経変性ならびに神経芽腫細胞粗面小胞体膜に存在するレトロウイルスは、粒子産生に必要でなかった。培養細胞における25nm粒子の列は、1968年に初めてスクレイピー感染脳のシナプス領域で報告され、続いて自然発症TSEおよびTSE実験モデルの多くに特定されたものと非常に類似している。この類似した分離ウイルス様粒子が内因性PrP抗体に対する反応性を示さず、感染性が高く、核酸-タンパク質複合体を破壊したときに感染性を失うことと合わせれば、当該25nm粒子が後期にPrP脳病変を誘発するTSEの原因ビリオンである可能性が高い。

| 報告企業の意見 | 今後の対応 |
|---|---|
| スクレイピー感染細胞およびクロイツフェルト・ヤコブ病病原体感染細胞では、TSEの原因と考えられる25nmウイルス様粒子が細胞内に生じるとの報告である。 | これまでの疫学研究等では、ヒトにおいて、血漿分画製剤を介してCJDを含む伝達性海綿状脳症(TSE)が伝播するという証拠はない。また異常プリオンが本製剤の製造工程で効果的に除去されるとの報告もあるが、輸血によりvCJDに感染する可能性が示唆されたことから、今後も情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

クロスエイトM250
クロスエイトM500
クロスエイトM1000

血液を原料とすることに由来する感染症伝播等
vCJD等の伝播のリスク

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# Cells infected with scrapie and Creutzfeldt–Jakob disease agents produce intracellular 25-nm virus-like particles

Laura Manuelidis, Zhoa-Xue Yu, Nuria Barquero, and Brian Mullins

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Notes:

# Cells infected with scrapie and Creutzfeldt–Jakob disease agents produce intracellular 25-nm virus-like particles

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Communicated by Sheldon Penman, Massachusetts Institute of Technology, Cambridge, MA, December 11, 2006 (received for review October 10, 2006)

**We had repeatedly found ~25-nm-diameter virus-like particles in highly infectious brain fractions with little prion protein (PrP), and therefore we searched for similar virus-like particles *in situ* in infected cell lines with high titers. Neuroblastoma cells infected with the 22L strain of scrapie as well as hypothalamic GT cells infected with the FU Creutzfeldt–Jakob disease agent, but not parallel mock controls, displayed dense 25-nm virus-like particles in orthogonal arrays. These particles had no relation to abnormal PrP amyloid *in situ*, nor were they labeled by PrP antibodies that faithfully recognized rough endoplasmic reticulum membranes and amyloid fibrils, the predicted sites of normal and pathological intracellular PrP. Additionally, phorbol ester stimulated the production of abnormal PrP gel bands by >5-fold in infected N2a + 22L cells, yet this did not increase either the number of virus-like arrays or the infectious titer of these cells. Thus, the 25-nm infection-associated particles could not be prions. Synaptic differentiation and neurodegeneration, as well as retroviruses that populate the rough endoplasmic reticulum of neuroblastoma cells, were not required for particle production. The 25-nm particle arrays in cultured cells strongly resembled those first described in 1968 in synaptic regions of scrapie-infected brain and subsequently identified in many natural and experimental TSEs. The high infectivity of comparable, isolated virus-like particles that show no intrinsic PrP by antibody labeling, combined with their loss of infectivity when nucleic acid–protein complexes are disrupted, make it likely that these 25-nm particles are the causal TSE virions that induce late-stage PrP brain pathology.**

infection | neuroectodermal cultures | virion ultrastructure | prion amyloid | retrovirus

It is often stated (1) that the transmissible spongiform encephalopathies (TSEs), such as sheep scrapie, human Creutzfeldt–Jakob disease (CJD), and bovine spongiform encephalopathy, are caused by an abnormal "infectious form" of the normal host prion protein (PrP). Abnormal PrP is visualized ultrastructurally as amyloid fibrils or as electrophoretic bands that are relatively resistant to proteinase K digestion (PrP-res). Although host PrP is required for TSE agent susceptibility, much as other specific host proteins are necessary for infection by a variety of viruses, abnormal PrP itself does not fulfill Koch's postulates for an infectious agent (reviewed in ref. 1). For example, it is not invariably present in highly infectious samples such as myeloid microglia (2), and it is not proportional to infectivity in subcellular fractions (3), infected cell cultures (4), or diseased brains (5, 6). Additionally, high PrP-expressing transgenic brains, as well as abnormal recombinant PrPs, have failed to show significant or reproducible infectivity (1). Moreover, despite 25 years of intense study, no "infectious conformation" has been structurally resolved, and the existence of many different TSE agent strains are incompatible with the speculation that different PrP conformations transmit or "encipher" strain specific information (7). Whereas TSE agents breed true despite passage through many different species and cell types with variant PrPs, abnormal PrP-res bands are species and cell-type specific (4). Abnormal PrP appears to be a relatively late pathological response to infection, rather than the causal agent itself (8, 9).

It is also claimed that there is no evidence for virus-like particles in TSEs (7). Yet there have been reports published by many different laboratories demonstrating very similar dense 20- to 35-nm virus-like particles in synaptic regions of TSE-infected brains. These particle arrays were shown first in experimental mouse scrapie in 1968 (10), in natural sheep scrapie by 1971 (11) and, subsequently, in primate and human CJD brain samples (12). These arrays were so virus-like in structure that they were considered to be papovaviruses (13). Similar particles in "paracrystalline" virus-like arrays also were identified in scrapie brain samples from Prusiner's laboratory and considered highly compatible with the 25-nm scrapie agent size as determined by membrane filtration of brain homogenates (14). By 1992, we obtained additional independent evidence for 25-nm spherical infectious particles by examining more purified agent preparations. Field flow sedimentation of highly infectious 120S brain fractions that first had been separated from the majority of abnormal (but uninfectious) PrP displayed virus-like 25- to 30-nm-diameter particles that behaved as 30-nm diameter control spheres; moreover, corresponding dense particles of the same diameter were identified by electron microscopy (EM) in this infectious preparation (15). Notably, these virus-like particles did not bind PrP antibodies, unlike the cosedimenting residual fluffy and amyloid fibril PrP (1). Treatment with low concentrations of SDS removed residual PrP from these particles but did not reduce their infectivity or size. In contrast, disruption of nucleic acid–protein complexes destroyed >99.5% their infectivity (16). Thus, there has always been good evidence for 25-nm virions in TSEs. A viral protein–nucleic acid complex is, moreover, the most parsimonious way to explain TSE strains and many other biologic properties of these agents (1).

With the establishment of high-titer CJD and scrapie agent-infected cell lines (4, 17), it became possible to test whether virus-like particles similar to those seen in more purified infectious brain preparations could be visualized in intact cells. Although these infected cell lines are of neural lineage, they do not develop synaptic structures and do not show the degenerative changes of TSE-infected brain. Thus, the presence of these virus-like particles in infected cells would show that synaptic and neurodegenerative processes are not required for their production. Because the infected cells used here also display large amounts of intracellular PrP amyloid without apparent ill effects (4), the relation of virus-like particles to the accumulation of

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Abbreviations: CB, cacodylate buffer; CJD, Creutzfeldt–Jakob disease; IAP, intracisternal A particle retrovirus; PMA, phorbol myristate acetate; PrP, prion protein; RER, rough endoplasmic reticulum; TSE, transmissible spongiform encephalopathy.

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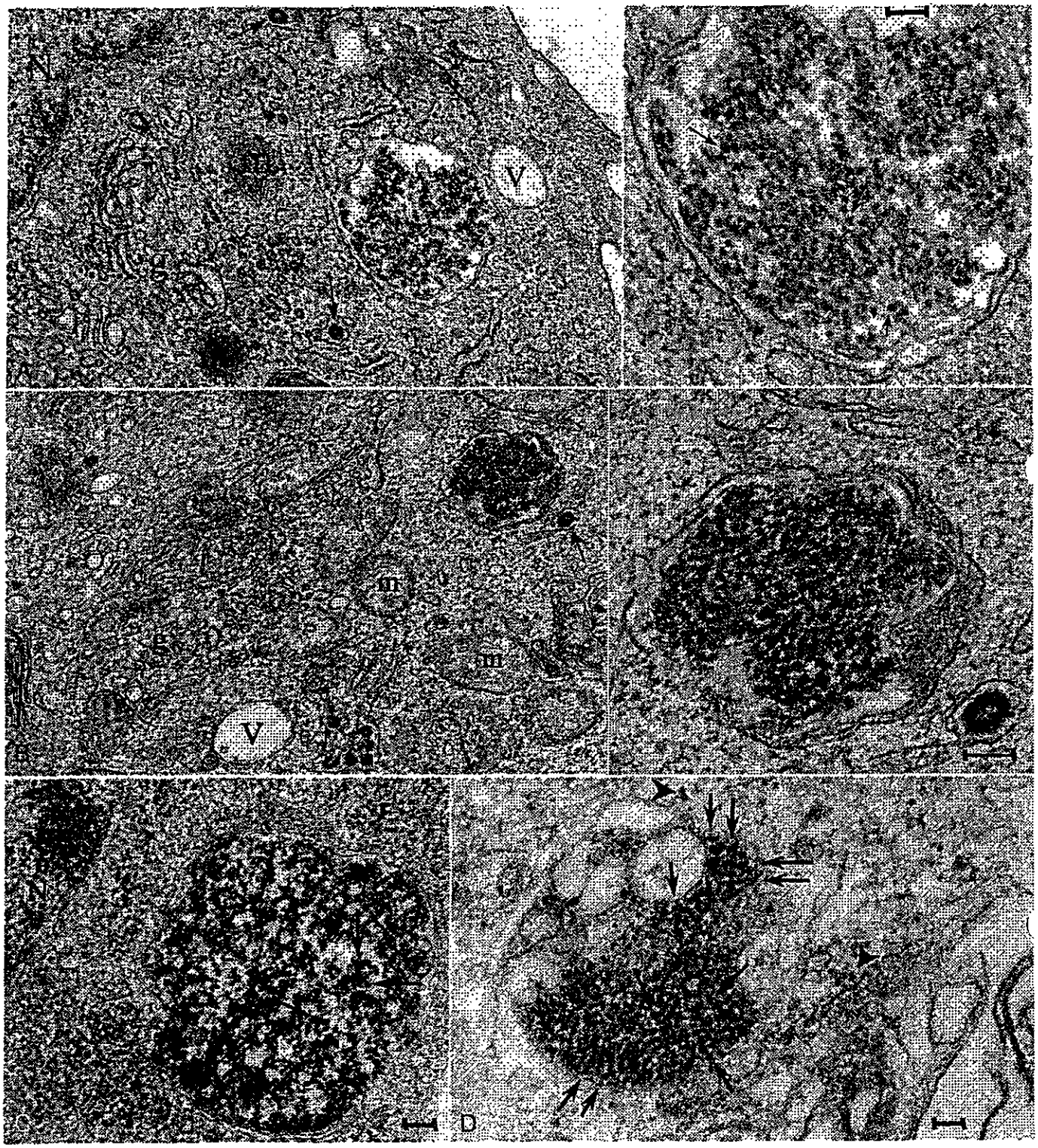


**Fig. 1.** Representative virus-like arrays of 25-nm particles in N2a + 22L (*A–C*) and in GT+FU-CJD (*D*) cells. (*A* and *B*) Particles in healthy cells treated with 100 ng/ml PMA for 5 days when PrP-res was markedly increased, with higher magnifications of the same array (*Right*). (*C*) From an N2a + 22L culture without PMA, processed in parallel. (*D*) A comparable virus-like array in a GT+FU-CJD cell. (*A Left*) The membrane-bound array is distant from the nucleus (N) and sits between a mitochondrion (m) and a vacuole (V). A 100-nm-diameter IAP retroviral is seen in an RER cistern (arrow), and Golgi membranes are at g. Arrows in the corresponding magnified image point to spherical dense particles of ≈25 nm; note regions of variable compaction of particles. (*B*) A characteristic cluster of IAP retroviruses is seen to the right of a vacuole (V) in addition to a TSE-packed particle array adjacent to an isolated IAP (arrow). Higher magnification shows that the paracrystalline particles have sharper outlines than adjacent ribosomes. (*C*) Without PMA, spherical 25-nm particles also line up in an orthogonal pattern in more compact regions of N2a + 22L cells. (*D*) Orthogonal or paracrystalline arrangement of equivalent dense 25-nm particles in cross-section (e.g., at arrows) in infected GT cells that produce no retroviral structures is shown; a few similar 25-nm particles are seen in an adjacent swollen RER cistern (arrowhead), and these may represent a different stage of array development (see *Results*). (Scale bars: 100 nm.)

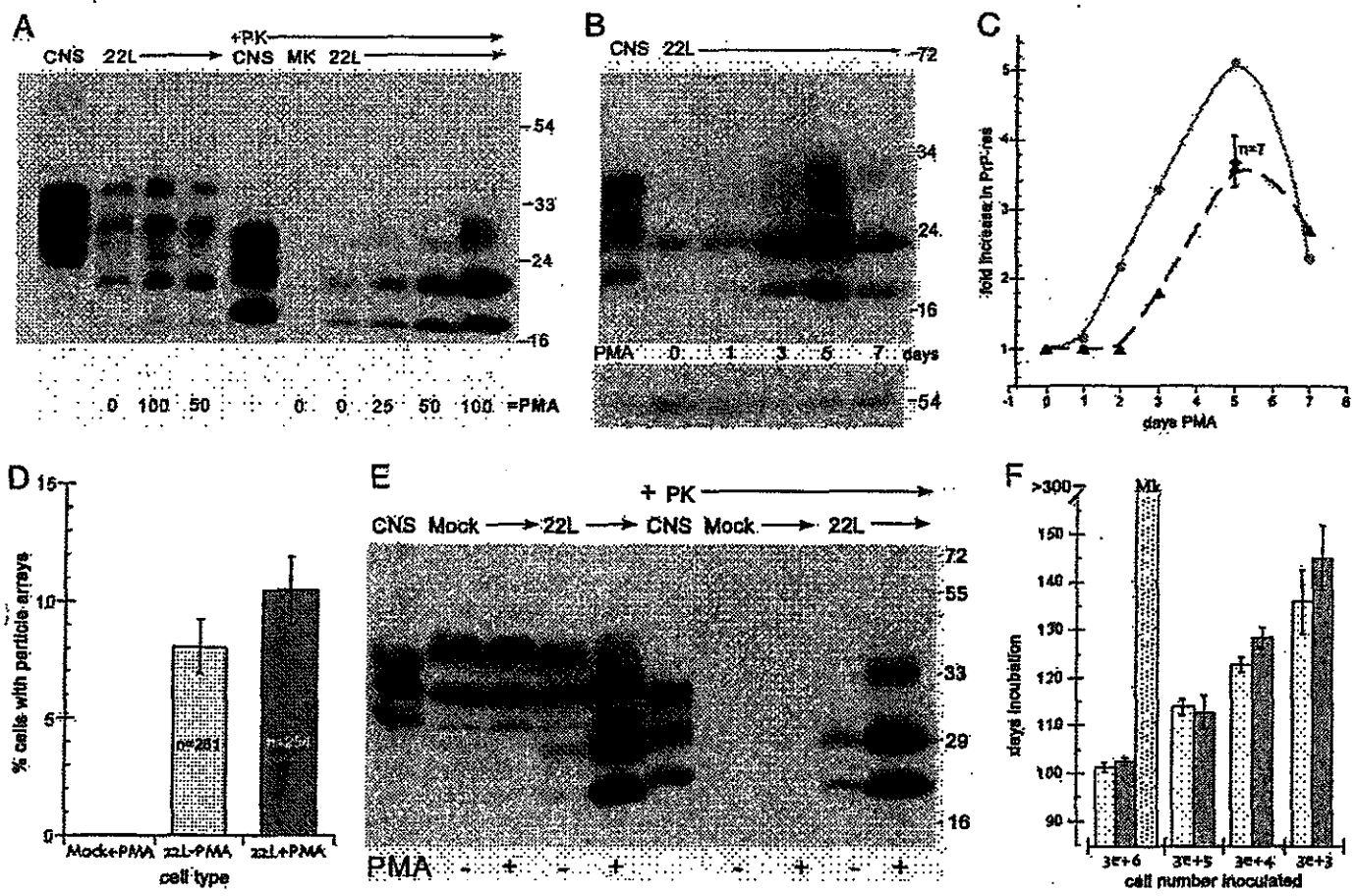


Fig. 2. Relationship of PrP-res, virus-like particles, and infectivity in N2a + 22L cells grown with and without PMA. (*A*) Dose–response from 0 to 100 ng/ml PMA for 5 days of PrP and corresponding PrP-res amounts (+Pk lanes of whole-cell lysates treated with proteinase K as described in ref. 4). Note the strong increase in PrP-res at 100 ng/ml. CNS is brain homogenate control. MK are mock-infected cells and show no PrP-res. (*B*) PrP-res during continuous treatment with 100 ng/ml for 1–7 days shows the strongest response at 5 days; cells were treated in parallel and collected at sequential times. (*C*) Quantitative analysis of the increase in PrP-res in seven independent experiments with 75–100 ng/ml PMA (dotted line). Note the reproducible rise at 5 days (± SEM). The gray line shows the PMA-induced PrP-res increase versus a parallel nondrug control (see *E*) in cells that were tested for infectivity. (*D*) Count of virus-like 25-nm particle arrays in PMA-treated mock (uninfected N2a) control cells and in N2a + 22L cells without (22L-PMA) and with (22L+PMA) the drug. More than 250 cells were counted by EM for each sample, and the inclusion of any questionably positive arrays makes the percentage of positives overestimated. Nevertheless, there is no significant increase in the number of particle arrays with PMA. (*E*) Western blot of an aliquot of the mock-infected, untreated 22L, and PMA-treated 22L cells inoculated in mice for infectivity titrations. The mock cells show no PrP-res (+PK lanes), and the PMA-treated cells show an obvious increase in both PrP and PrP-res compared with their untreated counterparts. All cell lanes loaded with equal amounts of protein and infected CNS show brain-specific pattern (4). (*F*) Incubation time after inoculation of serial dilutions of N2a + 22L cell homogenates grown without PMA (white bars) and with PMA (gray bars). A shorter incubation time indicates a higher infectious titer. Mock-infected cells (dotted bar) at high concentrations elicited no clinical signs or neuropathology.

abnormal PrP *in situ* and/or infection also could be evaluated. The following report demonstrates 25-nm virus-like arrays in two cell lines productively infected with either sheep-derived scrapie or human-derived CJD agents passaged in mice. Comparable particles were not found in uninfected controls. These data, along with other drug and PrP immunogold binding studies here, lend further credence to the actuality of a TSE virion that is structurally independent of pathological PrP in the intact cell.

## Results

We examined two different types of infected cells in detail. These represented two different types of TSE agent, a sheep-derived scrapie strain known as 22L and a mouse-passaged human CJD agent designated FU. The FU-CJD agent is an Asiatic isolate that is much more virulent in mice than the standard sporadic CJD agents isolated from patients in the Western hemisphere (6). Each of these TSE strains was propagated in different cell types. The 22L scrapie strain was propagated in neuroblastoma N2a58 cells (designated N2a + 22L) whereas the hypothalamic GT cell line was infected with FU-CJD (designated GT+FU-CJD). Thus, cell-specific and agent strain-specific influences could be monitored. Both infected cell types show abundant PrP amyloid fibrils by *in situ* immunocytochemistry (4) as well as high levels of PrP-res by Western blotting. As shown below, only the N2a cells (both uninfected and infected) produce endogenous intracisternal A type retroviral particles (IAPs). Thus, one could determine additionally whether these retroviral nucleic acid–protein complexes were linked to the production of 25-nm virus-like particles *in situ*. This was of interest because detergent-resistant IAP cores cosediment with TSE agents during subcellular fractionation of brain (18).

We initially searched for individual 25-nm dense round particles in regions of rough endoplasmic reticulum (RER) that had many developing IAPs and also looked for them in cytoplasmic regions with abundant PrP amyloid fibrils. Instead, we found dense virus-like 25-nm particles in paracrystalline arrays that were surrounded by a membrane. These bodies were separated from and morphologically different from both IAP clusters and




Fig. 3. Ultrasmall immunogold labeling (silver intensified) of PrP and PrP-res in N2a + 22L cells lightly fixed and then permeabilized *in situ* for antigen detection. (*Top*) A typical low-power image where many grains are on the plasma membrane (arrowhead) and dispersed in the cytoplasm. Nuclei (N) and clusters of IAP retroviruses (arrow) are not labeled. (*Middle*) Grains over PrP amyloid fibers in characteristic crisscrossing bundles (A) and also over RER membranes (arrows). Label was not seen over mitochondrial cristae (m) as representatively depicted. (*Bottom*) PrP does not localize over the 25-nm virus-like dense particles but can be associated with the outer RER membrane. (Scale bars: 100 nm.)

abnormal PrP fibrils. A striking feature, most consistent with a viral structure, was the uniform size of the particles in these arrays. This made them easily distinguished from lysosomes with heterogeneous inclusions, as well as other aggregates and dense bodies. The spherical 25-nm particles were variably packed in arrays and, in less compact regions, appeared connected by more lucent stalks. The dense viral spheres had no surrounding vesicular membranes. In compact regions, many of the particles lined up in orthogonal paracrystalline arrays at 90° to each other as in Fig. 1*C* (arrows). Although of similar density as adjacent ribosomes, the 25-nm virus-like particles displayed a more distinct outline and less fuzzy structure. The density of the TSE particles also was comparable with chromatin (a nucleic acid–protein structure), but particle arrays were spatially distant from nuclei, indicating their separate origin (Fig. 1 *A* and *C*). Particle arrays in the membrane-bound bodies overall measured from 0.3 to 1.0 $\mu m$ across. For internal reference, the IAPs seen in N2a cells are 100 nm in diameter. The C2a subclone of N2a + 22L with a 3- to 4-fold higher PrP-res content also displayed arrays that were morphologically indistinguishable from those in the parental line.

Because cells with virus-like inclusions had normal organelles and were healthy (Fig. 1 *A* and *B*), these virus-like inclusions could not be linked to degenerative processes. Their presence in cells without synapses also demonstrates they did not require elaboration of synaptic structures for their production. In addition, the same dense virus-like particle arrays also were present in GT+FU-CJD cells (Fig. 1*D*). Their presence in a different cell type that was infected by an unrelated TSE agent strain furthered the realization that these particles probably represent the common TSE agent structure. Although individual 25-nm particles outside of the membrane-bound arrays were very difficult to identify with certainty, they were present in some images of both N2a + 22L and GT+FU-CJD cells. Fig. 1*D* shows a few of these individual 25-nm particles that accumulated on or near an RER membrane (arrowhead). This region may represent either a nascent or a disintegrating array that is adjacent to a stack of several pathologic GT cell membranes. Virus-like particle arrays were not found in parallel mock N2a or GT cells.

To further evaluate the potential relationship of these TSE arrays to retroviral expression, PrP-res, and infectivity, we tested several chemical treatments that might alter these components. Phorbol myristate acetate (PMA) can stimulate transcription of viruses, including retroviruses and papovaviruses (19), and also can have other pleomorphic effects, including differentiation. We therefore added PMA to cells and were able to identify conditions that lead to an increase of PrP and PrP-res in infected but not mock N2a cells. Arrays from N2a cells were morphologically unchanged by treatment with PMA, as representatively shown in Fig. 1 *B* and *C*. Fig. 2*A* shows a representative dose–response curve of 22L infected and mock (MK) N2a cells with and without proteinase K (PK) digestion for PrP-res. There is a clear increase in PrP-res at 100 ng/ml for 5 days. No PrP-res is seen in the parallel MK control. Fig. 2*B* shows that the optimized time for this peak response was at 5 days, and the relative loads for each lane are shown by the 54-kDa tubulin internal standard. Fig. 2*C* graphs the quantitative changes in PrP-res in seven independent time course experiments with 75–100 ng/ml PMA (dashed lines). There is a reproducible ≈3.5-fold elevation in PrP-res at 5 days compared with drug-free

infected controls. The solid gray line shows the >5 fold increased PrP-res in the sample used to test whether there was a corresponding increase in titer with this high PrP-res.

We also counted the virus-like particle arrays in N2a + 22L cells that were treated with 100 ng/ml PMA for 5 days and then fixed for electron microscopy. To make sure that any potentially similar particles were not overlooked in mock cells, we included any possible similar virus-like arrays in the count, and hence the percentage of cells with these particles was somewhat overestimated. More than 250 cells each from mock, untreated, and PMA-treated preparations were counted for suspicious virus-like arrays (Fig. 2*D*). Only a single suspect particle could be found in >250 mock cells, and this single photographed inclusion had 7- to 15-nm-wide strings of dense material with no 20- to 35-nm-diameter spherical particles. Thus, no definitive similar virus-like arrays could be found in uninfected cells. As shown in Fig. 2*D*, there was also no significant elevation of particle arrays in the PMA-induced cells with high PrP-res. These molecular evaluations of PrP-res and particle counts were in accord with the spatial separation of virus-like particles and PrP amyloid fibrils, i.e., the two structures were mutually independent.

Ultrastructural studies of control and infected N2a cells also gave the impression of an increase in IAP particles with PMA treatment, but Western blots for IAP gag proteins by using IAP-specific antibodies as described in refs. 16 and 20 failed to confirm any major increase in retroviral proteins (data not shown). The PMA effects on PrP-res also appeared to be cell type and/or agent strain specific because GT+FU-CJD cells did not show any PrP-res increase of similar magnitude with 5 days of PMA at concentrations as high as 200 ng/ml. This high PMA level also did not educe any retroviral particles in GT+FU-CJD cells. Thus, the presence of TSE virus-like arrays in these GT+FU-CJD cells confirmed they were not dependent on, or derived from, retroviruses.

Aliquots of mock, untreated, and PMA-treated N2a cells were analyzed for both PrP-res (Fig. 2*E*) and for infectivity (Fig. 2*F*). In the Western blot of PrP and PrP-res, only the 22L infected cells showed a large increase in PrP-res with PMA treatment, and PMA did not induce either PrP or PrP-res in the uninfected controls (+PK lanes). The particle counts, but not the PrP-res data, predicted there would be no increase in infectivity with PMA stimulation. Thus, aliquots of these cells were inoculated intracerebrally to test whether the titer was increased with the PMA induced PrP-res. Fig. 2*F* shows these bioassays where serial dilutions of the untreated and the PMA-induced N2a + 22L cells were compared (white and gray bars, respectively). There was no significant shortening of the incubation time that would indicate higher infectivity in cells with the higher PrP-res. In fact, the more dilute inocula show that >5-fold increases in PrP-res accumulation yielded slightly longer incubation times. Additionally, the incubation times shown for the PMA-treated and control N2a + 22L cells represent an infectious titer of 1 $LD_{50}$ per ≈3 cells (see *Materials and Methods*). This is a very high level of infection and was comparable with the high titers of GT+FU-CJD cells. Significantly lower levels of infectivity are produced by other TSE cultures, including GT cells infected with 22L scrapie (4, 17). We are aware of no EM images of the 25-nm virus-like particles in other examined TSE cultures, and high levels of infectivity may be required to visualize these particles. Finally, control inoculations with high numbers of mock PMA-treated cells, as expected, yielded no signs of TSE disease in inoculated mice monitored for >300 days (Fig. 2*F*, dotted bar).

Because some abnormal PrP molecules might be in nonamyloid configurations, and normal PrP molecules are probably receptors for the infectious TSE agents (21), we used a third way to resolve whether there was an association of any PrP molecules with the 25-nm particles. Cells were lightly fixed, permeabilized, and then exposed *in situ* to PrP antibodies that detect both the normal and abnormal (N-truncated PrP-res) forms. After secondary ultrasmall gold-labeled detection and silver intensification, many silver grains were present over the plasma membrane (Fig. 3 *Top*, arrowheads) and dispersed through the cytoplasm, but were not over nuclei (N), mitochondria, or IAP clusters (at arrow). This pattern of PrP labeling was entirely consistent with the known residence of normal PrP. Densely packed amyloid fibers also were labeled as shown at A (Fig. 3 *Middle*), indicating good penetration of antibody and detector molecules. This higher magnification also shows that the cytoplasmic PrP label was over RER membranes, as would be expected from the known synthetic routes for a GPI-anchored protein such as PrP. No silver grains were seen over any of the 25-nm virus-like particles, but were sometimes found on the outer RER membrane that encircled the particle arrays (Fig. 3 *Bottom*). The above data contradict the notion that PrP, in any form, is an intrinsic component of the TSE 25-nm virus-like particles.

## Discussion

The intracellular 25-nm virus-like dense particles identified here were entirely consistent with a virion shape, size, and viral components as predicted from previous field flow sedimentation, HPLC, and molecular analyses of infectious particles that had been stripped of almost all PrP (16). Unlike host-encoded PrP, they were not detected in uninfected mock controls and, thus, were even more TSE-specific than PrP. They were also not part of the abnormal PrP amyloid, which is believed to be infectious. Furthermore, *in situ* labeling of intact cells, not subject to disruptive and artificial treatments of subcellular fractionation, also showed no association of particle arrays with PrP. Rather, individual 25-nm particles probably associate with PrP in the RER during their assembly, an observation that may explain why a small amount of PrP can remain associated with particles during fractionation. This particle–RER interaction also may initiate the pathological and self-propagating pathological amyloid of TSE disease, as proposed in ref. 21. Because IAP retroviral cores that attach to RER have the same density as the TSE agent (1.28 g/ml) and, therefore, copurify with the TSE agent, residual PrP in more purified infectious 25-nm particle preparations may derive from this source as well.

A recent field flow fractionation study of less pure scrapie brain preparations has reconfirmed the association of comparable ≈25-nm particles with infectivity. This study, however, concluded that these particles were constituted by aggregates of 14–28 PrP molecules, even though there were many other heterogeneous and uncharacterized molecules by silver staining in this peak infectious fraction (22). In contrast, the 25-nm spherical particles from our more purified brain preparations did not bind PrP antibodies (1). Thus, the interpretation of these particles as prions seems tenuous. The failure of PrP antibodies to label the 25-nm particles in cultured cells *in situ*, as well as the PMA-enhanced PrP-res experiments here, also support their nonprion nature. Because images of the particle arrays in our cultures were so virion-like, we researched the largely ignored nonprion literature, and realized (1), as Libersiki (12) had emphasized, how common and specific these particles are for TSE-infected brains. Furthermore, as in the above PrP-labeling studies, TSE-infected brain also showed no detectable PrP antibody binding to the 25-nm virus-like particles *in situ* (23). The strong uranyl positive density of these 25-nm particles in brain samples, their size and arrangement into similar orthogonal arrays connected by stalks, the RER membranes surrounding particles arrays, and the specificity of arrays for infected samples all underscored their similarity to the nonprion particles we independently identified here in two different infected cell lines that are free of degenerative morphology.

In the brain, the synaptic location of the TSE virus-like arrays also was consistent with the synaptic concentration of the TSE

infectious agent in synaptosomal fractions (24). Additionally, there is one published image of a comparable virus-like array in the perikaryon rather than in postsynaptic boutons (10), further indicating the similarities of the 25-nm particle arrays in brain and cultured cells. Although we did not observe tubular and electron lucent tubular-vesicular/membrane-like components of variable widths in our arrays, as sometimes described in the brain (12), tubular vesicular variants may be due to the elaboration of different components in mature neurons, different stages of particle development, or structures that are not integral to the much denser virus-like particles.

In summation, all of this data provides a clear, consistent, substantive, and logical alternative to the accepted prion hypothesis. The causative TSE agent is most consistent with an exogenous 25-nm virion without intrinsic host PrP. The stimulation of host innate immune responses by these agents, a complex set of molecular reactions that precedes the elaboration of pathologic PrP (9) and one that is not provoked by PrP-res itself (25), also point to a foreign pathogen rather than some unpredictably spontaneous mutation in the host's PrP without cause. The presence of these particles in many different species infected with a wide variety of TSE strains is in accord with Koch's first requirement (1). It is also improbable that an identical virus-like structure would be a contaminant or a secondary coincidental feature of all these different TSE models. Nevertheless, a more detailed molecular analysis of these particles will be required to substantiate their causal nature. Purification of these ≈25-nm particles from productive tissue cultures should be informative if the essential infectivity assays are performed systematically with parallel ultrastructural and molecular analyses. Animal titrations of infectivity are expensive and prolonged. However, sustained and reproducible infection of indicator GT cells by a variety of TSE agents already has shown that they can rapidly authenticate the presence of agent in disrupted samples as well as in living cells (4, 17). GT cells also may be used for testing infectivity of viral nucleic acids as well as PrP conformers. Rapid assays of infectivity in culture should facilitate the isolation of infectious particles from host components, and treatments that modify the production of these particles in culture may resolve further the infectious structure from the pathological disease processes it initiates.

## Materials and Methods

Neuroblastoma N2a58 cells infected with the 22L scrapie agent (26) at passage 230 were a gift of N. Nishida (Nagasaki University, Nagasaki, Japan) and were propagated for 50–150 additional passages at Yale, whereas mock N2a58 controls exposed to uninfected brain (4, 17) were passaged in parallel. The C2a subclone of 22L-infected N2a58 cells was also selected for its ≈4-fold higher PrP-res levels; this subclone yielded the same 25-nm particles and, thus, was not further discriminated here. The hypothalamic neuronal GT1.7 cell line that was infected with the FU-CJD agent consistently produced 1–10 $LD_{50}$ per cell by mouse bioassays (4) and repeatedly showed infectivity in a new rapid coculture assay (17). PMA (Sigma, St. Louis, MO) was applied to enhance PrP and PrP-res production in N2a + 22L cells as experimentally determined (see Fig. 2). For infectivity assays, cells were counted, disrupted, diluted, and inoculated intracerebrally into indicator mice as in ref. 4, and titers were calculated from standard incubation time to endpoint dilutions as described in ref. 21 by using 22L-infected brain homogenates inoculated in the same mouse genotype as the standard. Mice were assessed for strain-specific clinical signs and neuropathology by using a panel of standard antibodies (27). For Western blots, whole-cell lysates were analyzed with the M20 PrP antibody (4), and tubulin detection was used to confirm relative lane loads; calibrated chemiluminescent detections were done with an Alpha Innotech imager.

For EM, cells were fixed *in situ* in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4 (CB), for 1 h, harvested by scraping in CB with 1% fish gelatin, pelleted at 500 × *g* for 5 min and then at 3,000 × *g* for 10 min, washed in CB three times for 5 min, and exposed to 1% $OsO_4$ in CB for 1 h. They then were dehydrated and embedded in Epon 812. For immunolabeling, cells were fixed in 2% paraformaldehyde with 0.25% glutaraldehyde for 1 h, washed with CB, and permeabilized for 30 min at 25°C in CB with 2% normal rabbit serum and 0.2% saponin (Sigma). Cells were then exposed to goat M20 antibody (1:500) against the PrP C terminus (4) for 48 h at 4°C and 2 h at 25°C. After CB washes, cells were incubated at 25°C with 1:500 rabbit anti-goat IgG, washed, exposed to strepavidin ultrasmall gold (1:200; Electron Microscopy Sciences, Hatfield, PA), washed in CB and then $H_2O$, and enhanced with their silver kit for 15 min. After $H_2O$ washes, cells were osmified and embedded as above. Thin sections were stained with uranyl acetate and lead citrate; photographic negatives were scanned for reproduction.

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# 医薬品 研究報告 調査報告書

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**研究報告の概要** (125)

[1]英国保健省、月次vCJD・CJD統計、2007年1月8日時点
保健省は2007年1月8日、CJD患者数に関する最新情報を公表した。この中にはBSEと関連があると思われるvCJD患者も含まれる。内訳は以下の通り: vCJD患者:vCJD確定例における死亡患者:112名。vCJD可能性例における死亡患者(神経病理学的に未確定):46名。死亡患者総数:158名。vCJD患者-存命中:7名。vCJD確定例または可能性例総数:165名。2006年12月4日の月例統計以来、死亡患者総数には変化なく158名のままである。確定例または可能性例総数は1名増加して165名となった。このデータは英国におけるvCJD流行は減少しつつあるとする見解に一致する。死亡患者数のピークは2000年の28名であり、その後2001年に20名、2002年に17名、2003年に18名、2004年に9名、2005年に5名、2006年に5名と減少している。
[2]EUROCJD
EUROCJDの統計による全世界のCJD患者数の内訳は右表の通りである。

2006年12月時点でのデータ

| | 患者数累計 | 生存者 | 英国滞在6ヵ月以上 | 輸血による感染疑い |
|---|---|---|---|---|
| 英国 | 163 | 7 | 165 | 2 |
| フランス | 21 | 2 | 1 | 0 |
| アイルランド | 4 | 1 | 2 | 0 |
| イタリア | 1 | 0 | 0 | 0 |
| 米国 | 3 | 1 | 2 | 0 |
| カナダ | 1 | 0 | 1 | 0 |
| サウジアラビア | 1 | 1 | 0 | 0 |
| 日本 | 1 | 0 | 0 | 0 |
| オランダ | 2 | 0 | 0 | 0 |
| ポルトガル | 1 | 1 | 0 | 0 |
| スペイン | 1 | 0 | 0 | 0 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

**報告企業の意見**

英国保健省発表の統計によると、2007年1月8日時点で、vCJD確定例または可能性例総数は165名で、死亡患者総数には変化なく158名のままであり、英国におけるvCJD流行は減少しつつあるとする見解に一致するとの報告である。

**今後の対応**

日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

(52)

Back

**Archive Number** 20070108.0081
**Published Date** 08-JAN-2007
**Subject** PRO/AH/EDR> CJD (new var.) update 2007

CJD (NEW VARIANT) UPDATE 2007
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[The definition of the designations deaths, definite cases, probable vCJD cases, and the case definitions can be found by accessing the Department of Health website, or by reference to a previous ProMED-mail post in this thread (for example, CJD (new var.) - UK: update March 2002 20020305.3693).

Data on vCJD cases from other parts of the world are now included in these updates whenever available.

Also, data on other forms of CJD (sporadic, iatrogenic, familial and GSS) are now included when they have some relevance to the incidence and etiology of vCJD. - Mod.CP]

In this update:
[1] UK: Department of Health monthly vCJD and CJD statistics, Mon 8 Jan 2007
[2] EUROCJD data as of 8 Jan 2007
[3] National (US) Prion Disease Pathology Surveillance Center Data: 31 Dec 200
[4] Prion reduction by blood filtration
[5] Blood transfusion risk in France
[6] Blood transfusion risk in UK

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[1] UK: Department of Health monthly vCJD and CJD statistics, Mon 8 Jan 2007
Date: Mon 8 Jan 2007
From: ProMED-mail <promed@promedmail.org>
Source: UK Department of Health, Monthly Creutzfeldt-Jakob Disease Statistics [edited]
<http://www.gnn.gov.uk/environment/fullDetail.asp?ReleaseID=254733&NewsAreaID=

The Department of Health is today [Mon 8 Jan 2007] issuing the latest information about the numbers of known cases of Creutzfeldt-Jakob disease. This includes cases of variant Creutzfeldt-Jakob disease [abbreviated in ProMED-mail as CJD (new var.) or vCJD], the form of the disease thought to be linked to BSE (bovine spongiform encephalopathy).

Definite and probable CJD cases in the UK, as of Fri 5 Jan 2007
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Summary of vCJD cases - deaths
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Deaths from definite vCJD (confirmed): 112
Deaths from probable vCJD (without neuropathological confirmation): 46
Deaths from probable vCJD (neuropathological confirmation pending): 0
Number of deaths from definite or probable vCJD (as above): 158

Summary of vCJD cases - alive
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Number of probable vCJD cases still alive: 7

Total
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Number of definite or probable vCJD (dead and alive): 165

(The next table will be published on Mon 5 Feb 2007).

Since the previous monthly statistics were released on Mon 4 Dec 2006, the total number of deaths from definite vCJD remains unchanged and stands at 158. The overall total number of definite or probable vCJD cases (dead and alive) has increased by one (surviving) individual and becomes 165.

These data are consistent with the view that the vCJD outbreak in the UK is in decline. The peak number of deaths was 28 in the year 2000, followed by 20 in 2001, 17 in 2002, 18 in 2003, 9 in 2004, 5 in 2005, and 5 in 2006.

Totals for all types of CJD cases in the UK in 2006
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As of Fri 5 Jan 2007, in the UK in the year 2006, there were 106 referrals, 53 deaths from sporadic CJD, 5 from familial CJD, 5 from variant CJD, 3 from GSS and one from iatrogenic CJD.

During the period from 1995, when vCJD was 1st diagnosed, up to the present, there have been 953 deaths from all forms of CJD including the 158 deaths attributable to definite or probable vCJD.

[These data are accessible via
<http://www.gnn.gov.uk/environment/fullDetail.asp?ReleaseID=254733&NewsAreaID=

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ProMED-mail
<promed@promedmail.org>

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[2] EUROCJD data as of 8 Jan 2007
Date: Sun 31 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: EUROCJD [edited]
<http://www.eurocjd.ed.ac.uk/vcjdworldeuro.htm>

The European And Allied Countries Collaborative Study Group of CJD (EUCJD)
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This website includes information from 2 projects funded by the European Commission. The EUROCJD project started in 1993 and compares data from national registries in Australia, Austria, Canada, France, Germany, Italy, the Netherlands, Slovakia, Spain, Switzerland and the UK. The NEUROCJD project started in 1998 after the European Union Council recommended that epidemiological surveillance of CJD should be extended to all member states. The member states involved in this project are Belgium, Denmark, Finland, Greece, Iceland, Ireland, Israel, Norway and Portugal. Both projects are coordinated from the U.K. National CJD Surveillance Unit based in Edinburgh.

Current data as at December 2006*
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Country / Total No. of Primary cases (No. alive) / Cumulative residence in UK (>6 months) / Secondary transmission by blood transfusion

United Kingdom / 163 (7) / 165 / 2 (0)
France / 21 (2) / 1 / 0
Republic of Ireland / 4 (1) / 2 / 0
Italy / 1 (0) / 0 / 0
USA / 3 (1*) / 2 / 0 / 0

Canada / 1 (0) / 1 / 0
Saudi Arabia / 1 (1) / 0 / 0
Japan / 1** (0) / 0 / 0
Netherlands / 2 (0) / 0 / 0
Portugal / 1 (1) / 0 / 0
Spain / 1 (0) / 0 / 0

Total / 198 (12) / - / 2

Footnotes
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* The 3rd US patient with vCJD was born and raised in Saudi Arabia and has lived permanently in the United States since 2005. According to the US case report, the patient was most likely infected as a child when living in Saudi Arabia.
** The case from Japan had resided in the UK for 24 days in the period 1980-1996.

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ProMED-mail
<promed@promedmail.org>

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[3] National (US) Prion Disease Pathology Surveillance Center Data: 31 Dec 200
Date: Mon 8 Nov 2006
From: ProMED-mail <promed@promedmail.org>
Source: CJD Surveillance, National Prion Disease Pathology Surveillance Center [edited]
<http://www.cjdsurveillance.com/resources-casereport.html>

[The Prion Disease Surveillance Data remains essentially unchanged. Previously, the Moderator noted an apparent inconsistency between the national data and a figure circulated by the Virginia Department of Health. The Virginia Department of Health/CDC report cited above lists 3 cases -- 2 cases probably contracted in the U.K. -- and the young adult described above who was born and raised in Saudi Arabia. The EUROCJD data for October 2006 in part (2) above also lists 2 U.S. cases, both assumed to have been contracted in the U.K. The U.S. National Prion Disease Pathology Surveillance Center Data published on 8 Nov 2006, on the other hand, lists only a single U.S. case believed to have been contracted in the U.K. The following clarification has been provided by Ermias Belay of the CDC/CCID/NCZVED]:

The inconsistency noted by Mod.CP is appropriate, but it stems from the fact that the EUROCJD data is for October 2006 and was not updated to include the 3rd U.S. vCJD case in a Saudi man announced by CDC in November 2006. The data from the U.S. National Prion Disease Pathology Surveillance Center lists only cases confirmed by the center. The only U.S. vCJD case tested at the pathology center is the 1st U.S. case. The 2nd case was tested and confirmed in England and the 3rd case was tested and confirmed by UCSF. Incidentally, the 1st Saudi vCJD case was tested and confirmed by the U.S. pathology center, but the patient was never in the United States (hence not attributed to the U.S.); brain biopsy tissue was shipped to the U.S. by Saudi physicians.

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ProMED-mail
<promed@promedmail.org>

[ProMED-mail thanks Ermias Belay for clarifying the situation. - Mod.CP]

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[4] Prion reduction by blood filtration

Date: Fri 22 Dec 2006
From: ProMED-mail <promed@promedmail.org>
Source: BBC News online [edited]
<http://news.bbc.co.uk/1/hi/health/6200107.stm>

Scientists hail blood filtration technique

Scientists have found a way to remove disease-causing proteins from infected animal blood, which they hope may fight the human form of mad cow disease. UK and US researchers identified a molecule which removed the prion proteins from blood infected with scrapie, the Lancet reports. Scrapie, which can affect sheep, is related to variant CJD. It is hoped the technique could also treat human blood. But a blood transfusion expert said it 1st had to be properly assessed. Prion diseases, including bovine spongiform encephalopathy (BSE) -- or mad cow disease -- and variant Creutzfeldt-Jakob Disease (vCJD), are fatal neurodegenerative diseases that occur after years of incubation with no apparent symptoms.

In the UK, 3 people have died from vCJD after receiving blood from donors who later went on to develop the disease. Globally, there have been 200 cases of vCJD reported, 164 of which were in the UK. The researchers, including scientists from Cambridge University, suggest removing the infectious ability of prions -- mis-formed proteins -- might be one of the best ways of reducing the risk of vCJD transmission through blood. They screened millions of molecules and found that one, called L13, binds prion protein (PrP), removing it from the blood.

The team checked this by passing 500 ml of scrapie-infected hamster blood through a filter that removes white blood cells, something which is done to all blood donated from transfusion in the UK. When they injected the blood into 99 hamsters, 15 became infected with scrapie. But when they passed treated blood through devices containing the removal molecule and injected 96 hamsters with it, they found that none became infected. L13 was also found to bind to PrP from human infections of vCJD, suggesting that it may also remove prion infectivity from human blood. Dr. Robert Rohwer, from the Veterans Affairs Medical Center, at the University of Maryland, Baltimore, who was involved in the study, said: "Removal of vCJD infectivity by adsorption gets around the extremely difficult problem of detecting the very low concentrations of these agents in blood, especially during the long asymptomatic period when people donate.

Writing in the Lancet, Marc Turner of the Edinburgh Blood Transfusion Centre, said there were concerns about how best to check whether the technology worked, as there is nothing to compare it against. There are also worries that passing blood through filters may cause blood to be caught up in the device and therefore lost, or could alter the blood's properties. He added that: "The UK and Irish Blood Services have produced quality, efficacy and operational specifications for such filters and are considering an assessment program that will include independent investigation of efficacy and clinical safety studies. Until these technologies can be clinically and operationally assessed, the best protection against the uncertain risk of transfusion-associated prion disease remains in

ensuring that blood products are used only if needed and that the uncertainties surrounding potential risks are communicated effectively to patients and to the public at large."

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[The Lancet paper which is the subject of this report is entitled: "Reduction in infectivity of endogenous transmissible spongiform encephalopathies present in blood by adsorption to selective affinity resins," authored by Luisa Gregor and 8 others, <http://www.thelancet.com/journals/lancet/article/PIIS0140673606698978/abstrac

The Summary reads as follow:
"Background: Transmissible spongiform encephalopathies (TSE) can be contracted through blood transfusion. Selective adsorption of the causative agent from donated blood might be one of the best ways of managing this risk. In our study, affinity resin L13, which reduces brain-derived infectivity spiked into human red blood cell concentrate by around 4 log10ID50, and its equivalent, L13A, produced on a manufacturing scale, were assessed for their ability to remove TSE infectivity endogenously present in blood.

Methods: 500 ml of scrapie-infected hamster whole blood was leucoreduced at full scale before passage through the affinity resins. Infectivity of whole blood, leucoreduced whole blood (challenge), and the recovered blood from each flow-through was measured by limiting dilution titration.

Findings: Leucoreduction removed 72 percent of input infectivity. 15 of 99 animals were infected by the challenge, whereas none of the 96 or 100 animals inoculated with the final flow-throughs from either resin developed the disease after 540 days. The limit of detection of the bioassay was 0□2 infectious doses per ml. The overall reduction of the challenge infectivity was more than 1.22 log10ID. The results showed removal of endogenous TSE infectivity from leucoreduced whole blood by affinity ligands. The same resins adsorb normal and abnormal prion protein from human infections with variant, sporadic, and familial Creutzfeldt-Jakob disease, in the presence of blood components.

Interpretation: TSE affinity ligands, when incorporated into appropriate devices, can be used to mitigate the risks from TSE-infected blood, blood products, and other materials exposed to TSE infectivity." - Mod.CP]

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[5] Blood transfusion risk in France
Date: Tue 26 Dec 2006
From: Terry Singeltary <flounder9@verizon.net>
Source: Transfusion Clinique et Biologique, .11.003, 2006 [edited]
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VN7-4MMP63G-2&_use

Transfusion risk analysis with regard to vCJD in France
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(By M. Martin, J.-F. Legrasa, E. Pouchola and J.-H. Trouvina (DEMEB, departement de l'evaluation des produits biologiques, 143-147, boulevard A.-France, 93285 Saint-Denis cedex, France)

ABSTRACT: The latest updates (February 2004 and February 2005) of the analysis of the risk of transmission of the agent of Creutzfeldt-Jakob disease (CJD) by blood and blood products in France 1st reported in 2000, were triggered by the 2 cases of probable transmission of variant CJD (vCJD) by transfusion reported in the UK and the notification of 2 French cases of vCJD who had been blood donors on several occasions before clinical onset. Even though some figures of the quantitative assumption used in the risk analysis have been modified since 2000, the conclusion as regards the risk for blood cellular component is considered unchanged: it can be assumed that one unit of labile blood products will contain more than one infectious unit if the donor is incubating the disease. Therefore, the residual risk of receiving by transfusion one infectious blood unit is dependent on the prevalence of subjects incubating the disease in the blood donor population. For this particular aspect, the expected number of clinical vCJD cases to occur in France has been lowered since 2000. However, the worst-case scenario of 300 cases in the next 60 years has been maintained in the risk analysis, leading to the hypothesis that one blood donor per 120 000 could be infectious. In conclusion, the risk of getting one infectious blood unit is considered probable to a level of 1/120 000, but the benefit outweighs the risk if the use of transfusion is restricted to well justified indications and if patients are informed a priori and a posteriori.

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[6] Blood transfusion risk in UK
Date: Tue 26 Dec 2006
From: Terry Singeltary <flounder9@verizon.net>
Source: Transfusion Clinique et Biologique,.11.007, 2006 [edited]
<http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VN7-4MMP63G-5&_use

vCJD and blood transfusion: risk assessment in the United Kingdom
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(By S.A. Dobra, a, and P.G. Bennettam Department of Health, Wellington House, 133-155 Waterloo Rood, London SE1 8UG, UK)

ABSTRACT: The risk of vCJD transmission via blood transfusion depends on potential levels of infectivity, recipients' exposure to infected donors and individual susceptibility. On infectivity, SEAC (the UK's main scientific advisory committee on TSEs), has published an updated position statement. Based on animal models, this suggests that infectivity is split roughly equally between leucocytes and plasma, with negligible levels directly associated with red cells or platelets. Risk assessments are now therefore based on the amounts of plasma and leucocytes within each component as transfused. Recipients' exposure to infection depends critically on the prevalence of infection in the population. This remains unknown, so a range of assumptions must still be considered. A further consideration is the likelihood of any infected donors' blood being infective. Those infected in the primary outbreak will now have been incubating vCJD for 10-25 years. Current thinking is that blood may be more infective later in the incubation period. This reinforces the case for a precautionary approach to transmission risks,

despite the small number of incidents seen so
far. Exposure will also depend on how many donors
contributed components to a given individual.
Recent work has shown that more patients receive
large numbers of units than previously thought.
These highly-transfused patients are a particular
cause for concern. The current precautionary
assumption is of all recipients being susceptible
to infection by transfusion, though incubation periods may differ markedly.

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[see also:
CJD (new var.), blood transfusion risk 20061208.3468
CJD, transmission risk - Canada (ON) 20061207.3457
CJD (new var.) update 2006 (12) 20061205.3431
CJD (new var.) update 2006 (11) 20061106.3190
CJD (new var.) update 2006 (10) 20061002.2820
CJD (new var.) update 2006 (09) 20060904.2519
CJD (new var.) update 2006 (08) 20060807.2207
CJD (new var.) update 2006 (07) 20060703.1831
CJD (new var.) - Netherlands: 2nd case 20060623.1741
CJD (new var.) update 2006 (06) 20060605.1566
CJD (new var.) update 2006 (05) 20060508.1332
CJD (new var.) update 2006 (04) 20060404.1005
CJD (new var.) update 2006 (03) 20060306.0728
CJD (new var.) - UK: 3rd transfusion-related case 20060209.0432
CJD (new var.) update 2006 (02) 20060206.0386
CJD (new var.) update 2006 (01) 20060111.0101
CJD (new var.) update 2006 20060111.0101
2005
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CJD (new var.) update 2005 (12) 20051209.3547
CJD (new var.) update 2005 (11) 20051108.3270
CJD (new var.) update 2005 (10) 20051006.2916
CJD (new var.) update 2005 (09) 20050905.2627
CJD (new var.) update 2005 (08) 20050801.2237
CJD (new var.) update 2005 (07) 20050703.1889
CJD (new var.) update 2005 (06) 20050607.1584
CJD (new var.) update 2005 (05) 20050505.1243
CJD (new var.) update 2005 (04) 20050405.0982
CJD (new var.) update 2005 (03) 20050308.0687
CJD (new var.) update 2005 (02) 20050211.0467
CJD (new var.) - UK: update 2005 (01) 20050111.0095
2004
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CJD, genetic susceptibility 20041112.3064
CJD (new var.) - UK: update 2004 (14) 20041206.3242
CJD (new var.) - UK: update 2004 (13) 20041103.2977
CJD (new var.) - UK: update 2004 (12) 20041023.2871
CJD (new var.) - UK: update 2004 (11) 20041008.2758
CJD (new var.) - UK: update 2004 (10) 20040909.2518
CJD (new var.) - UK: update 2004 (09) 20040809.2199
CJD (new var.) - UK: update 2004 (08) 20040806.2150
CJD (new var.) - UK: update 2004 (07) 20040706.1807
CJD (new var.) - UK: update 2004 (06) 20040608.1535
CJD (new var.) - UK: update 2004 (05) 20040510.1262
CJD (new var.) - UK: update 2004 (04) 20040406.0937
CJD (new var.) - UK: update 2004 (03) 20040314.0713
CJD (new var.) - UK: update 2004 (02) 20040202.0400
CJD (new var.) - UK: update 2004 (01) 20040106.0064
CJD (new var.) - France: 8th case 20041022.2864
CJD (new var.) - France: 9th case 20041123.3138
CJD (new var.), blood supply - UK 20040318.0758
CJD (new var.), carrier frequency study - UK 20040521.1365
2003
---
CJD (new var.) - UK: update 2003 (13) 20031216.3072
CJD (new var.) - UK: update 2003 (01) 20030108.0057
2002
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CJD (new var.) - UK: update Dec 2002 20021207.5997
CJD (new var.) - UK: update Jan 2002 20020111.3223

# 医薬品
# 医薬部外品　研究報告　調査報告書
# 化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 12 月 8 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Atypical transmissible spongiform encephlopathies (TSEs) in ruminants<br>Baron, T. et al.,<br>Vaccine, in press, available on line at www.sciencedirect.com on 13 Nov. 2006 | 公表国<br>フランス | |
| 販売名 (企業名) | | | | | | |

**研究報告の概要**

この報告文献は反芻動物における感染性海綿状脳症 (TSE) －特にヒツジにおける異型 TSE－に焦点をおいた現在の知見の概要である。宿主異常タンパク質であるプリオンタンパク ($PrP^{res}$／正常プリオンタンパクと異なりプロテアーゼ消化耐性をもつ) の感染組織への蓄積は TSE の重要な特徴である。ウエスタン・ブロット法と免疫組織化学的分析により，ウシ海綿状脳症 (BSE) 感染動物の脳から分離した $PrP^{res}$ とスクレイピー感染したヒツジから分離した $PrP^{res}$ の識別は可能である。これらの技術により，フランスでは，BSE が確認された $PrP^{res}$ と類似した分子構造を持つヤギ TSE 自然発生症例を識別出来た。マウスにおける感染実験では，ヤギから分離した感染因子と BSE 病原体の区別は不可能であった。また，ヒツジにおける TSE 自然発生症例のその他の分離株では，ウエスタン・ブロット法で検出された特徴と実験的に誘発された BSE 症例での $PrP^{res}$ の特徴が非常に類似していることが確認された。ヒツジ PrP を過剰発現させたトランスジェニックマウスでの予備感染実験の結果では，異なった TSE 病原体「株」の存在が示唆された。ヨーロッパ諸国の中では，長年，小型反芻動物からの異型 TSE 分離株の存在が知られている。これらの症例では，病原性プリオンタンパクは脳内部での特異的な分布に加えて，プロテアーゼ感受性の変化や新規の電気泳動パターンを示していた。そのうえ，この内の数例は，従来のスクレイピー又は BSE に耐性のあるプリオン遺伝子型をもつヒツジで発現していた。これらの TSE はヒツジ PrP 過剰発現マウスに感染することが確認された。また，ヨーロッパでは畜牛でも異型 TSE が発生している。これらの症例では，従来の BSE によって発症したものと比較すると，$PrP^{res}$ の糖鎖付加パターンが変化していた。ウシとマウス両方のモデルでは，アミロイド斑として知られている新型の病変と同様に海綿状脳障害独特の分布とも関連していた。反芻動物におけるこのような異型 TSE の起源はまだ不明あるが，ヒトにおけるクロイツフェルト・ヤコブ病の「散発性」と「変異性」と類似している。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本実験結果から，TSE に関与するプリオンは当初考えられていた以上に多様性に富み，またヒトへの感染はプリオンの種類によって異なることが示唆された。これは，ウシ由来製品を含有する血漿分画製剤の製造工程におけるプリオン除去工程を評価する際に考慮が必要となる可能性がある。 | 弊社の血漿分画製剤の製造工程において，これまでに同定されている種々のウシプリオンに対してプリオン除去工程は効果的であることが確認されている。<br>現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。 |

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# Atypical transmissible spongiform encephalopathies (TSEs) in ruminants

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**Abstract**

Transmissible spongiform encephalopathies (TSEs) are associated with the accumulation in infected tissues of a disease-associated form of a host-encoded protein, the prion protein (PrP). Contrary to the normal form of the protein, this form of PrP is partially resistant to protease digestion ($PrP^{res}$). Detailed characterisation of $PrP^{res}$ has been intensively investigated in recent years to try and decipher the diversity of TSEs in human and animals. This considerably and unexpectedly enlarged our knowledge about such diseases in ruminants. Previously, such a diversity was essentially shown by the demonstration that scrapie from sheep and goats could have different biological behaviours following transmission of the disease in mice, unlike bovine spongiform encephalopathy from cattle (BSE) which showed a distinct and unique behaviour. The properties of the BSE agent were also demonstrated to be very stable, following transmission to a variety of different species. Molecular studies of $PrP^{res}$, followed by transmission studies to mice, gave the first evidence for the accidental transmission of the BSE agent to humans where it induced a variant form of the fatal Creutzfeldt-Jakob disease (CJD) and also to different animal species including a goat in France. This last case was found among a few unusual cases of TSEs in small ruminants that showed some molecular similarities with BSE and which are currently under investigation by transmission studies in mice. The application of the molecular methods to characterise $PrP^{res}$ has most recently led to the unexpected discovery of deviant BSE forms in a few affected cattle in Europe and in the United States, which raises the question of a possible different origin at least of some cases of BSE in cattle. Finally, considerable numbers of a new TSE form in small ruminants, referred to as "atypical scrapie" or "Nor98", have meanwhile been identified in most European countries by TSE rapid testing using an assay which recognizes also comparatively less PK resistant $PrP^{res}$.
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*Keywords:* Atypical; BSE; Scrapie; Prion; Strain

## 1. Introduction

Transmissible spongiform encephalopathies (TSEs) are fatal neuro-degenerative diseases, affecting both human (Creutzfeldt-Jakob disease; CJD) and animals, including mainly sheep and goats (scrapie), deer and elk (chronic wasting disease; CWD) and cattle (bovine spongiform encephalopathy; BSE). The exact nature of the infectious agent involved in the transmission of these diseases remains controversial. However, a central event in their pathogenesis is the accumulation in infected tissues of an abnormal form of a host-encoded protein, the prion protein (PrP) [1,2]. Whereas the normal cellular protein is fully sensitive to proteases ($PrP^{sen}$), the disease-associated prion protein ($PrP^{d}$) is only partly degraded ($PrP^{res}$), its amino-terminal end being removed.

Experimental transmissions of these diseases, especially that of scrapie from small ruminants, revealed a biological diversity of the involved infectious agent, reminiscent of "strains" among classical infectious agents, like viruses [3]. This has been mainly described in genetically defined inbred wild-type mice, by the different features of the disease between different strains, including differences in the

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incubation periods and in the distribution of brain lesions; these features remained generally remarkably stable following serial passages of a given strain in a given mouse model [4,5].

Distinct and specific features of the $PrP^{res}$ protein, as characterised in most studies by Western blot methods, have also been found in mice or in hamsters infected with different biological strains of TSE agents, suggesting that biological properties of the infectious agent might be enciphered in the conformation of the disease-associated form of the prion protein [6–12].

## 2. Search for the possible presence of BSE in small ruminants with natural TSEs

Characterisation of the infectious agent associated with BSE showed unique features, with characteristic incubation periods of the disease, as well as a defined distribution and nature of brain lesions, following transmission of the disease in wild-type mouse lines [13–16]. Studies of brain lesions essentially involved the histological analysis of the distribution and intensity of spongiform changes in precise neuro-anatomical sites, but can also rely on the neuro-anatomical mapping of $PrP^{d}$ by PET-blot method and/or immunohistochemistry [15–17]. Such features assessed by bioassay in mice remained unchanged following transmission of the BSE agent after passages from cattle to other species [5,14,15,17].

Western blot analyses of $PrP^{res}$ accumulating in the brains of BSE-infected animals and humans have also demonstrated specific molecular features, compared to most other cases of prion diseases found in the same species, with a lower apparent molecular mass of the unglycosylated $PrP^{res}$ moiety and very high proportions of diglycosylated $PrP^{res}$ [18,19] (Fig. 1). This lower apparent molecular mass in BSE has been linked to a particular proteinase K cleavage, leading to a possible discrimination from most scrapie sources by differential $PrP^{res}$ labelling with different antibodies against different regions of the PrP protein; this was indeed associated with a specific loss of $PrP^{res}$ immunoreactivity with certain monoclonal antibodies that recognize a particular region of the protein which is specifically digested in BSE (for instance P4 or 12B2 antibodies recognising the 93-WGQGG-97 amino-acids sequence in the sheep PrP) [8,20–23]. Similar molecular discrimination between BSE and scrapie in small ruminants was also obtained using immunohistochemical methods for mapping of $PrP^{d}$ cellular cleavage [24–27].

Such findings have allowed the research of a possible transmission of the BSE agent in natural field conditions in animals, such as in sheep and goats. This recently led to the identification in France of a first case of TSE in a goat (CH636) with $PrP^{res}$ molecular properties similar to that found in BSE [28]. Characterisation of the infectious agent by transmission studies in mice confirmed, by analysis of the incubation periods of the disease and of the distribution of brain lesions, that it was undistinguishable from the cattle BSE agent. More recently, molecular similarities between a natural TSE case identified in a goat in the UK were also reported, although bioassay has not yet been completed to confirm the isolation of the BSE agent in this case [25].

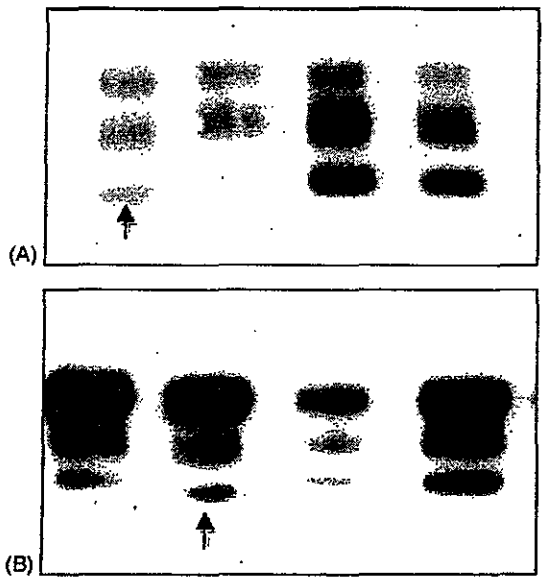


Fig. 1. Western blot detection of $PrP^{res}$ in BSE (arrow) compared to three scrapie sources, in C57Bl/6 mice (panel A) or in sheep (panel B). The lower band corresponding to the unglycosylated $PrP^{res}$ shows a lower apparent molecular mass in BSE-infected animals.

However, as early as 1999, it was shown that some scrapie isolates could also show very close $PrP^{res}$ Western blot patterns to that found in experimental ovine BSE [29]. This was demonstrated in an historical British scrapie isolate, CH1641 that had been used in scrapie transmission experiments in sheep. In this case, although subtle differences with ovine BSE might be found by Western blot [21,22], the apparent molecular masses of unglycosylated $PrP^{res}$ were hardly distinguishable between CH1641 and ovine BSE. Immunohistochemical approach could show that, in sheep infected with the CH1641 isolate, $PrP^{d}$ in neurones differs from BSE in having an even longer segment of the N-terminus digested by intracellular enzymes [26]. The biological properties of the infectious agent associated with this CH1641 experimental scrapie isolate are clearly distinct from BSE, since, contrary to BSE, it consistently failed to be transmitted to wild-type mice [30].

A few other TSEs isolates with close $PrP^{res}$ molecular properties by Western blot to that found in ovine BSE have been identified, although these showed different features in immunohistochemistry compared to experimental ovine BSE [27]. Such isolates are currently under investigation by transmission in mice. First results led to puzzling observations following transmission in a transgenic mouse line (TgOv-PrP4) that over-express the ovine PrP protein (A136 R154 Q171 sequence) [31] and that was shown to faithfully repro-

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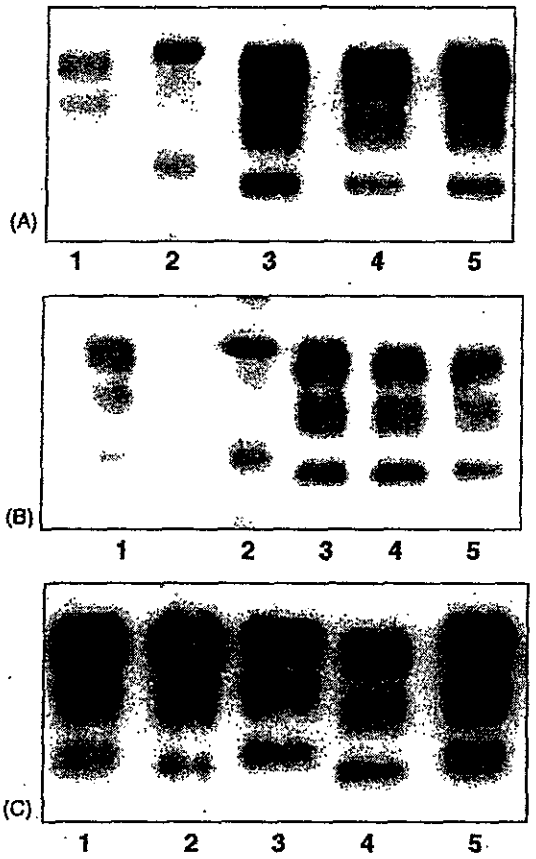


Fig. 2. Western blot detection of $PrP^{res}$ in ovine transgenic mice (TgOvPrP4 mouse line) infected with a natural BSE-in-goat sample (CH636)(panel A, lanes 3–5) [28], CH1641 scrapie experimental isolate (panel B, lanes 3–5) or O104 field scrapie TSE isolate (panel C, lanes 3–5) [27]. Controls included experimental scrapie strain C506M3 in lane 1 and BSE in panel C, lane 2 as described in Ref. [22]. Molecular weight markers in panels A and B, lane 2, with the 29.0 and 20.1 kDa markers. Note the lower apparent molecular mass of unglycosylated $PrP^{res}$ in all three mice infected with BSE or CH1641, by comparison to that from a C506M3-infected mouse, as well as the very intense labelling of the upper, diglycosylated, band in BSE-infected mice. In mice infected with the O104 isolate, two distinct $PrP^{res}$ types, with different apparent molecular masses of unglycosylated $PrP^{res}$, can be detected according to the mouse brain analysed.

duce the distinct $PrP^{res}$ molecular features from different scrapie and BSE sources [22] (Fig. 2). They indeed revealed that, contrary to what was previously observed with usual scrapie cases or with ovine BSE, $PrP^{res}$ molecular features in mice were variable between individual mice, with two $PrP^{res}$ forms differing by the apparent molecular masses of the unglycosylated form. Although biological characterisation of the infectious agent still remains to be achieved from such unusual TSE cases, such results could suggest the possible presence of distinct strains in such isolates.

## 3. Atypical scrapie/Nor98 in small ruminants

In EU countries, active surveillance of TSEs in small ruminants implemented since 2002 has allowed to identify rapidly a number of unusual isolates, notably in France, Germany and Great Britain [32,33], in sheep and in goats. These "atypical" isolates were characterised by (i) discrepancies in results obtained by different rapid diagnostic tests based on $PrP^{res}$ detection and (ii) difficulties in confirmation by OIE recommended diagnostic methods. Overall results of biochemical studies suggested a lower protease resistance of a $PrP^{res}$ form that might explain such discrepancies between diagnosis tests.

In fact, discrepancies between diagnosis tests and a lower protease resistance of $PrP^{res}$ had already been reported in the first description of some unusual scrapie isolates, which had been identified as early as 1998 in Norway as Nor98 scrapie isolates [34]. In these Nor98 isolates, $PrP^{d}$, although never present at very high levels, was found to be more abundant in the cerebellum and in the cortex than in the brain stem, in contrast with classical scrapie or ovine BSE. Also, Western blot analyses, when positive, showed a complex electrophoretic pattern including an unusual band of low apparent molecular mass (≈10–12 kDa), while in classical scrapie $PrP^{res}$ is detected as three bands between 18 and 29 kDa corresponding to the un-, mono- and di-glycosylated $PrP^{res}$ forms (Fig. 3) [35]. This $PrP^{res}$ fragment was recently found to be cleaved at both N- and C-terminal ends of the protein, in contrast with the only N-terminal cleavage found in classical scrapie and BSE [36]. The hypothesis that such atypical cases could only be artefacts and not authentic TSEs was discussed for a long time, especially for those cases found in France, Germany and Great Britain which were first identified by discrepancies between different rapid tests and/or confirmatory diagnosis methods [32]. Importantly all these last cases were identified following attempts to identify $PrP^{res}$ in the brain stem, which is the most appropriate sample for classical scrapie or BSE.

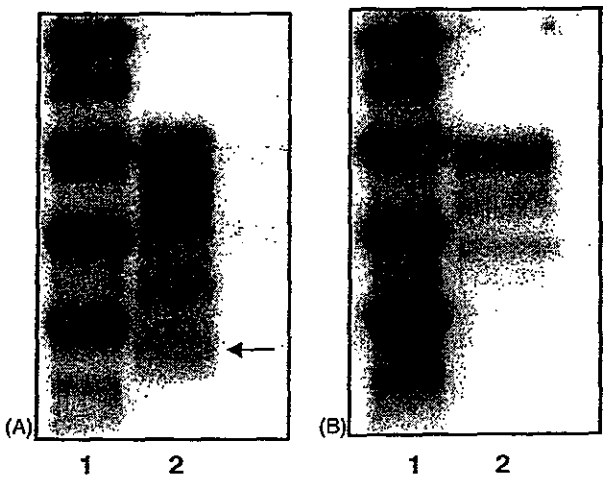


Fig. 3. Western blot detection of $PrP^{res}$ in sheep with atypical (A) or classical (B) scrapie. Molecular weight markers in lane 1 showing the 58.1, 39.8, 29.0, 20.1 and 14.3 kDa markers. Note the complex electrophoretic pattern in atypical scrapie with at least five major bands, including a band at ≈10–12 kDa (arrow), while three bands between 18 and 30 kDa are present in classical scrapie.

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Furthermore, a number of these cases were identified in sheep with genotypes of the prion gene associated with high resistance to classical scrapie or BSE, such as those with the A136 H154 Q171 or A136 R154 R171 [32,33]. Some cases were also found in sheep homozygous for the A136 R154 R171 allele [33,37], which are considered as the most resistant to classical scrapie. Interestingly, Nor98 isolates had also been described in sheep with such "resistant" genotypes [34,35]. More recently, a strong association of Nor98 isolates with a leucine/phenylalanine polymorphism at codon 141 was also recognised in sheep with the A136 R154 Q171 allele [38].

The possibility that such cases were not genuine TSEs was definitively ruled out by the successful transmission of 10 French atypical isolates and three Norwegian Nor98 cases to transgenic mice over-expressing the ovine PrP ($V_{136}$ $R_{154}$ $Q_{171}$ allele) [39]. These successful transmissions also revealed striking similarities between French atypical TSE cases and Nor98, which all showed consistent incubation periods, distribution of brain lesions and $PrP^{res}$ molecular features in mice. Remarkably, by Western blot, $PrP^{res}$ in both atypical scrapie and Nor98 infected mice showed a unique electrophoretic pattern from all the analysed sources, with notably a small $PrP^{res}$ fragment of 10–12 kDa, quite distinct from the three bands (18–29 kDa) $PrP^{res}$ pattern observed in classical scrapie and in BSE infected mice. Similar results regarding the successful transmission and $PrP^{res}$ Western blot features were more recently obtained in a transgenic mouse line (TgOvPrP4) [31] expressing the ovine A136 R154 Q171 PrP protein.

Overall, these data, showing major differences with classical scrapie and BSE in a number of features of the disease, raise important questions about the origin of such cases, that have now been recognised with a high incidence and in an increasing number of countries [40–44]. Major uncertainties remain especially regarding the pathogenesis of the disease in these cases, as well as concerning their potential transmissibility within the affected species and to other species.

## 4. Atypical TSEs recognised in cattle

Molecular characterisation of $PrP^{res}$ has also allowed to recognize atypical cases of TSEs in cattle. BSE in cattle was indeed considered to be a disease with unique features, in relation with its origin as a food borne epidemic [45,46]. The origin of the infectious agent involved in BSE remains unknown, but infectious meat-and-bone meal in which a TSE agent would have been recycled is considered as the origin of the epidemic. However, it was found that a few cattle showed by Western blot molecular features of $PrP^{res}$ distinct from the majority of cattle with typical BSE (Fig. 4).

In a study of three French cattle, $PrP^{res}$ with a higher apparent molecular mass of its unglycosylated form was reported (H-type) [47]. This was associated with strong labelling by a particular antibody, P4, contrary to typical BSE, showing that in these atypical H-type isolates, the PrP region recognised by P4 antibody was preserved following proteinase K digestion. Such cases were also recognised in other countries such as Germany [48]. Recently, transmission of a disease in wild-type (C57Bl/6) mice following intra-cerebral inoculation from such cases has been reported, which showed that the distinct molecular features were maintained in mice [49]. First histopathological data also showed a different distribution of spongiform lesions, and the presence of amyloid plaques, which are not found in this mouse model with typical BSE.

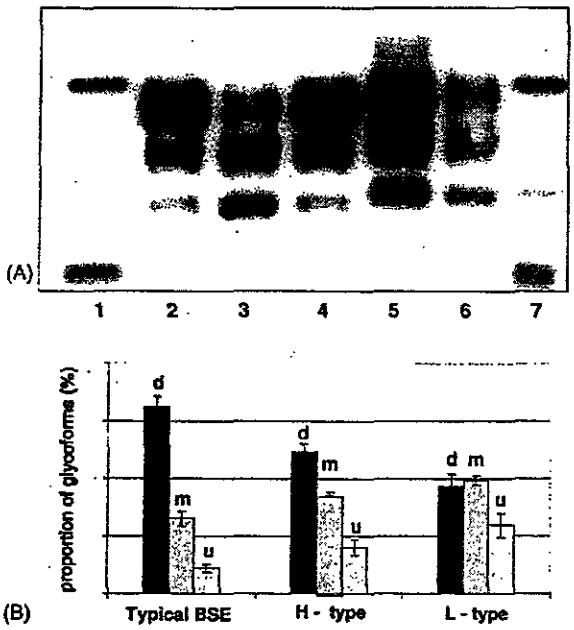


Fig. 4. (A) Western blot detection of $PrP^{res}$ in cattle with atypical forms of BSE, including the H-type (lanes 5–6) and L-type (lane 3), in comparison with typical BSE (panel A, lanes 2 and 4). Molecular weight markers in lanes 1 and 7 showing the 29.0, 20.1 and 14.3 kDa markers. (B) Proportions of the three PrPres glycoforms, diglycosylated (d), monoglycosylated (m) or unglycosylated (u), showing the decreased levels of diglycosylated PrP in atypical cases of BSE, especially in the L-type, compared to typical BSE.

Another study described two cattle in Italy in which $PrP^{res}$ showed a lower apparent molecular mass of the unglycosylated $PrP^{res}$ (L-type isolates) but the most striking difference with BSE was that of the strongly decreased levels of the diglycosylated $PrP^{res}$ form which is prominent in typical BSE [50]. Study of these two cases in Italy also reported significant differences in the distribution of brain lesions, but also in the nature of lesions, with the presence of amyloid plaques; this led to propose the term of bovine amyloidotic spongiform encephalopathy (BASE) for this novel pathology. Such cases have also been reported in some other countries, such as Poland, Germany and France [48,51,52]. Interestingly, such a L-type case in Germany could be transmitted by intra-cerebral inoculation in a bovine transgenic mouse model, in which incubation period of the disease was significantly shorter than that found with typical BSE [48]. Molecular analyses showed similarly decreased proportions of diglycosylated $PrP^{res}$ to those initially described in cat-

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tle, but also distinct distribution of brain lesions compared to mice infected with typical BSE.

The origin of such deviant features in cattle TSEs remains unexplained yet. However, several hypotheses can be considered including (i) a major change of the BSE agent, (ii) infection of such animals with another source of TSE agent, such as scrapie from sheep and goats, (iii) the existence of a previously unrecognised form of TSE in this species. Each of these hypotheses can lead to some comments: (i) a phenotypic change of the BSE is unlikely, since a number of experimental studies have shown the remarkable stability of the BSE agent, including following cross-species transmission; such an phenomenon can however not be fully excluded since such a phenotypic switch has already been documented in a study of BSE transmission in a particular wild-type mouse line (SJL)([53], (ii) contamination by scrapie has been considered as a major hypothesis for the origin of the BSE epidemic in cattle, which could have been triggered initially by the recycling via meat-and-bone meal of such an agent after transmission in cattle, (iii) the situation in cattle, with two distinct phenotypes of TSEs beside the typical form of BSE, is reminiscent of the situation in human with distinct forms of "sporadic" Creutzfeldt-Jakob disease in addition to the so-called variant form of Creutzfeldt-Jakob disease which is caused by the BSE agent [14,18].

## 5. Conclusion

The precise role of the prion protein in the transmissibility of TSEs remains debated, especially regarding the possibility that this protein might be the sole constituent of the infectious agent ("protein-only" hypothesis). The existence of "strains" with distinct and specific transmissible biological properties, at least in mouse experimental models, has largely contributed to this controversy. Recent experimental data have, however, reinforced the possibility that strain properties might be associated with the disease-associated prion protein. Molecular studies of this protein have recently allowed to identify several forms of TSEs which were previously unrecognised, in cattle and in small ruminants. These findings were also a consequence of the substantial increase of TSE surveillance, especially in Europe. While the significance of these forms of diseases remains to be more precisely established, they already question about their possible occurrence, in other parts of the world, including in regions such as New Zealand or Australia, which were considered to be free of animal TSEs so far. It is possible that some of these newly recognised forms of TSEs are similar to "sporadic" forms of neuro-degenerative diseases, including most of the cases of Creutzfeldt-Jakob disease in humans and, in all these situations, the occurrence of such "sporadic" cases would raise the question of the origin of such diseases.

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| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 1980 年代後半に英国で乳牛におけるウシ海綿状脳症(BSE)が発生して以来，牛乳及び乳製品の安全性は重大な懸念事項であった。予防として，専門家らは罹患ウシ及びそのミルクがヒトの食物連鎖の中に入るべきではないということを提言してきた。本文献では，種々の動物のミルク及び初乳中のプリオンに関する研究から，ミルクの安全性に対する証拠を検討している。スクレイピー(ヒツジのプリオン病)の母子伝播がヒツジ及びヤギにおいて認められているが，スクレイピーに感染しているヒツジから得られたミルク，初乳又は乳腺組織を異なる経路を介して実験的にマウスに注射したが，症状を誘導することはできなかった。ウシにおける BSE の母子伝播に関する大規模試験によって，母子伝播は感染動物の 10％を占めることが立証された。しかしながら，マウスで実施された実験から，感染ウシからその仔牛への BSE 伝播にミルクが関与している可能性は否定された。1997 年に，ミルク，初乳及び乳腺組織は，BSE 感染性が検出されない組織として分類された。ヒトにおいて，母乳による伝播を含む vCJD の母子伝播に関する疫学的な証拠は存在しない。結論として，潜在的 BSE 感染ウシから得られたミルクを摂取することによって vCJD に罹患するリスクは実際にはごく僅かであることが示唆された。 | BYL-2007-0274 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| これまでに報告されていたミルクやウシ初乳の摂取によるプリオン感染リスクはごく僅かであることが，本研究結果によって再確認された。 | 現時点で新たな安全対策上の措置を講じる必要はないと考える。 |

# Bovine spongiform encephalopathy and the safety of milk from Canadian dairy cattle

M. G. TYSHENKO

**The detection of bovine spongiform encephalopathy (BSE) in beef cattle closed Canadian beef export markets to 30 countries, including the USA, with devastating financial losses. The detection and confirmation of the fifth and seventh BSE-infected animals but first infected dairy cows extended the problem of risk management to Canadian dairy farmers. As the public are concerned about the safety not only of beef but also of milk and milk products that may contain disease-causing prions, this review examines the evidence for the safety of milk from studies on prions in milk or colostrum and their vertical and lateral transmission in various animal systems. The evidence indicates that the risk of contracting new variant Creutzfeldt-Jakob disease through the consumption of milk is negligible.**

THE outbreaks of bovine spongiform encephalopathy (BSE) in cattle, and new variant Creutzfeldt-Jakob disease (vCJD) in human beings were spread by the practice in many countries of feeding ruminant-derived materials to other ruminants, and the movement of infected live cattle, meat products and contaminated feeds. The outbreak in the UK raised questions about whether various animal-derived food products, including dairy products, posed a risk to the health of human beings. For cattle entering the human food chain, restrictions were placed on high-risk materials, including the brain, spinal column and certain organs that have the highest titres of infective material. In Canada, beef consumers became aware of prion disease in domestic beef cattle after the detection of the first BSE-positive cow in 2003 (Coulthart and others 2003). The focus of research on BSE and the safety of human health have centred around beef, as many Canadians consume large quantities of meat in their diet. However, the detection of one imported and two domestic dairy cows infected with BSE have raised public concerns about the safety of milk and milk products in North America (Centers for Disease Control and Prevention 2004).

Although BSE-infected dairy cattle have been detected only recently in North America, the question of the safety of milk from dairy cattle is not new. During the outbreaks in the UK from the late 1980s to 2005, many dairy cattle became infected with BSE. Dairy cattle were used for milk production and for processing into foods such as ground beef, sausages and cuts of meat. The concern over prions in milk resulted in it being one of the products first targeted for a risk analysis. The Southwood Working Party was the first expert panel convened in the UK to assess the level of risk. On the basis of previous experience with scrapie, a similar spongiform encephalopathy affecting sheep, the risk of transmission to human beings was believed to be remote. However, owing to the degree of uncertainty, the expert panel recommended that affected animals and their milk should not enter the human food chain (Smith 2004).

The British government enforced the recommendation to ban milk from BSE-infected cattle for both human consumption (Pratt 1991). In 1991, the Ministry of Agriculture, Fisheries and Food was given powers under section 35(1) to seize, destroy and dispose of animal carcases suspected of having died from BSE. The powers under sections 1 and 8 were also used to protect human health by ordering the disposal of milk from cows affected by BSE (BSE Inquiry 2000a).

Milk is produced by a lymphatic-type tissue, and as prions can be detected in lymphatic tissue there is the possibility that milk may carry disease-inducing prions (Aguzzi 2006). Infected dairy cows may yield milk before they show clinical signs of BSE, and it is known that prions accumulate for years in infected animals before clinical signs appear. When infected but apparently healthy cows are milked, their milk is mixed in a collection tank with milk from other healthy cows; if the disease-causing prion is present in this milk it would contaminate the entire batch, and could repeatedly contaminate milk from each subsequent batch. Thus, an infected dairy cow's milk may enter the human food chain and be consumed over many months or years.

Furthermore, it is known that the infectious prions are extremely resistant to heat and resist normal sterilisation processes (Brown and others 2000), so pasteurisation does not completely destroy them (Fernandez Garcia and others 2004). As a result, the public continue to be concerned about the safety of milk and other dairy products in relation to BSE infectivity (Trevitt and Singh 2003, Vetrugno 2004). This paper reviews the results of experiments that have been conducted in various animal systems to try to answer questions regarding milk safety.

## STUDIES IN SHEEP

### Maternal (vertical) transmission – evidence from sheep and scrapie

At the beginning of the BSE outbreak in the UK, it was hypothesised that the source of the infection was scrapie in sheep, and it was estimated that scrapie affected one-third of all British sheep flocks (Morgan and others 1990). It had also been assumed that the disease in sheep was transmitted genetically, but this could not explain the widespread prevalence of the disease (Dickinson and others 1965, 1966). Studies proving the occurrence of maternal transmission provided a partial explanation for the distribution of scrapie within sheep flocks. The studies of maternal transmission also tested the possibility that offspring might become infected from their mother's milk. Mammary gland tissue from two scrapie-affected ewes and colostrum samples taken at parturition from six high-risk ewes were used to prepare prion material for intracerebral injection into groups of 10 mice. No transmission of infectivity was detected in the mice (Hadlow and others 1982).

### Mouse bioassay for scrapie

Although maternal transmission of scrapie had been observed in sheep and goats, it had not been induced in laboratory mice infected experimentally with scrapie. Various membranes and fluids were tested for infectivity, including fetal membranes, faeces, urine, saliva, milk, semen and colostrum, and different methods of infecting the mice were used, including intracerebral, subcutaneous and intravenous injections or oral dosing. None of the mice infected with any of these materials, by any of these routes, developed scrapie (Hourrigan and others 1979).

*Veterinary Record* (2007) 160, 215-218

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TABLE 1: Evidence in various animal systems and known transmission of disease-causing prions by detection in milk or colostrum, vertical transmission or lateral transmission

| Animal system | Prion detection in milk or colostrum | Vertical transmission (mother to offspring) | Lateral transmission (iatrogenic, placental, ingestion, other tissue contamination) |
|---|---|---|---|
| Human (kuru) | No* (Ridley 1995), untested but inferred | No* (Ridley 1995) | Yes* (Ridley 1995), previous practice of eating contaminated brain |
| Human (vCJD) | No (Vetrugno 2004)<br>Yes† (Tamai and others 1992), results retracted | No (Baker and Ridley 1996) | Yes (Williams 2006), human by blood transfusion and iatrogenic transmission |
| Sheep and goat (BSE/scrapie) | No (Hadlow and others 1982), inferred by mouse bioassay<br>Yes (Aguzzi 2006), detection in milk sediment in mammary tissue (Pattison and Millson 1962) | No‡ (Foster and others 2004), challenged with cattle prion<br>Yes (Lacey and Dealler 1994, Caplazi and others 2004), challenged with sheep prion (Bellworthy and others 2005), natural BSE transmission in sheep flock | Yes (Ryder and others 2004), contaminated feed (Foster and others 1993, 2001) |
| Cattle (BSE) | No (Vetrugno 2004, Everest and others 2006) | Yes§ (Donelly and others 1997a) | Yes (Dealler and Lacey 1991), contaminated feed |
| Mouse bioassay (BSE) | No (Taylor and others 1995), orally infected with BSE cows' milk | No# (Taguchi and others 1993) | Yes (Maignien and others 1999), experimentally infected |
| Mouse bioassay (knockout transgenic expressing cattle BSE prion) | No (Castilla and others 2005a) | Yes (Castilla and others 2005b) | Yes (Castilla and others 2005b), experimentally infected |

* Incidence of kuru decreased significantly in people born after cannibalism ceased in the mid-1950s; no children born after 1959 to any of the women who had or subsequently developed kuru have themselves developed the disease

† One case was reported in 1992 of a 38-year-old pregnant woman with sporadic Creutzfeldt-Jakob disease (CJD) whose colostrum was found to be infected when injected intracerebrally into mice. However, following further morphological examination of fixed mouse brain by immunohistochemistry for $PrP^{Sc}$, the Japanese authorities concluded that the published results were invalid because no spongiform change or $PrP^{Sc}$ was found on first passage from human colostrum to mice. The mouse brain from the second passage (mouse to mouse) did show spongiform change and $PrP^{Sc}$ but this was not attributed to transmission from the colostrum

‡ Cheviot ewes challenged orally with bovine spongiform encephalopathy (BSE) cattle brain produced lambs of various PrP genotypes; of 72 surviving to more than 30 months of age, 29 were of the most susceptible PrP genotype; none of the progeny showed any signs of disease

§ Data reveal a significantly higher risk of disease in calves born to BSE-affected dams, with the risk being greatest in calves born after the onset of clinical signs of disease in the dam

# No transmission of CJD in mice was observed in 75 offspring born to CJD agent-inoculated females or to normal females mated with inoculated males, or in 19 normal offspring maintained by foster nursing with the inoculated mothers

The attempt to compare intracerebral, intravenous and oral doses complicated the studies. The infectious dose of prions injected intracerebrally was markedly smaller than the infectious doses of prions given parenterally, intravenously or orally. The dose of prions needed to cause an infection may also vary with the strain of prion, the physical composition of the aggregated prions, the source tissue, and the genetic susceptibility of the host animal (Erdtmann and Sivitz 2003).

## STUDIES IN CATTLE

### Maternal (vertical) transmission – evidence from cattle and BSE – the maternal cohort study in cattle, 1989 to 1997

The most important research undertaken to investigate maternal transmission in cattle was the cohort study carried out between 1989 and 1997 at the Central Veterinary Laboratory in the UK. In this study, 300 calves from affected dams were matched with 300 control calves from unaffected dams to follow the subsequent development of BSE. One weakness of the study was that calves in both the groups had already been exposed to infected cattle feed; a second weakness was that there could have been significant differences in genotypes between the subjects and controls that might have affected the outcome. However, it was believed that the size and design of the study, and the fact that both groups had had similar exposures to contaminated feed, would minimise the former weakness. The results showed that BSE occurred in 14 per cent of the offspring of the affected dams but in only 4·3 per cent of the offspring of the unaffected dams; the difference between the control and experimental groups was statistically significant (Wilesmith and others 1997).

A secondary analysis of the results of the cohort study by other groups independently confirmed that maternal transmission was responsible for up to 10 per cent of affected cattle, but that this rate would not have been adequate to maintain the BSE epidemic (Donnelly and others 1997a, b, Ferguson and others 1997, Gore and others 1997). The experimentally established value of 10 per cent remains the accepted contribution for the maternal transmission of BSE in cattle populations.

### Mouse bioassay for BSE

The infectivity of BSE is usually demonstrated experimentally. A bioassay is used to show that BSE can be transmitted between animals, in which suspect tissue is inoculated into a target species or laboratory animal. Such research concluded that the transfer of BSE from affected cows to their calves in milk could be excluded as a route of transmission.

In one experiment, milk from BSE-affected cows was given orally to mice; a cohort of 275 mice consumed an average of 300 ml of milk per mouse over a period of 40 days. Groups of mice were also given either an intracerebral injection of 0·02 ml milk or an intraperitoneal injection of 0·1 ml milk. The mice were observed for two years during which none of them developed signs of disease and none had the characteristic neurological lesions postmortem (Taylor and others 1995). Although these results support the hypothesis that milk does not transmit infective prions, there are doubts about the relative transmissibility of the disease from cows to mice owing to the potential species barrier. The results of a similar experiment, in which calves were infected intracerebrally or orally with milk from infected cows, thus obviating the species barrier, have not been reported (Wells and others 1998).

Experiments to resolve the infectivity of cattle tissues in the mouse bioassay suggested that several non-neural tissues known to be infective for scrapie were not infective for BSE. The results supported the idea that the mouse bioassay was probably not sufficiently sensitive to detect BSE infectivity across the species barrier, and that the pathogenesis of BSE differed markedly from the pathogenesis of scrapie. The comparative bioassay has shown that the mouse bioassay is

approximately 1000 times less sensitive than a similar assay in cattle (BSE Inquiry 2000b).

### Lateral (close association) transmission of BSE – mouse and transgenic mouse models

The infectivity of the placenta was studied for its potential involvement in the lateral transmission of BSE from mother to offspring. Studies in sheep had shown that placental transmission is an endemic route of infection of lambs with scrapie (Brotherston and others 1968). However, feeding mice with milk, udder, supramammary lymph nodes, placenta and fetal membranes from BSE-infected cattle failed to transmit the disease (Middleton and Barlow 1993); again, these results were considered inconclusive owing to the effect of the species barrier between mice and cattle.

As a method for overcoming the species barrier, researchers created a prion 'knockout' mouse that had its endogenous prion gene disrupted or made inactive. The knockout mice did not make any prion proteins and did not become diseased when exposed to infective prions. The knockout mouse line was then made transgenic by adding the cattle prion gene to their genome, so that the mice would express cattle prions; BSE can be transmitted to these mice much more easily and with much shorter incubation times. These transgenic mice have provided a more sensitive bioassay by eliminating the species barrier and by more closely approximating the aetiology of the disease in cattle (Scott and others 1997).

In a transgenic mouse line expressing bovine prion protein (boTg), BSE prions were detected in the brains of pups born to mothers infected intracerebrally when they had been mated close to the clinical phase of the disease. However, the intracerebral inoculation of transgenic boTg mice with infected milk was ineffective, suggesting that mother to offspring transmission involved other tissues as carriers of infected prions (Castilla and others 2005a, b).

Table 1 gives a summary of various animal systems and knowledge of the transmission of BSE in milk (direct evidence), or from mother to offspring and lateral transmission (indirect evidence).

## DISCUSSION

The transmission of BSE in milk has not been reported from any BSE-affected country and there is similarly no evidence of the transmission of scrapie or the human spongiform encephalopathies (CJD and kuru) in milk. The lack of evidence suggests that milk either does not readily transmit infective prions (Haltia and others 1979, van Duijn and others 1998, Vetrugno 2004), or that its infectivity is too low.

The risk of acquiring vCJD from drinking milk appears to be much lower than from eating other specified risk materials, beef or other tissues from BSE-infected cattle. Data to support the very low risk attributable to contaminated milk comes from the suckler herd study completed in the UK in 1997, which was designed specifically to investigate milk safety. Investigations with suckling cows under practical conditions provided no evidence that BSE was transmitted from the milk of infected cows to their calves (Wilesmith and others 1997).

In 1997, the Scientific Steering Committee of the European Commission categorised BSE risk materials into four categories. Colostrum, milk and mammary gland tissues were classified in category 4, as tissues in which infectivity could not be detected. At that time, the term 'not detected' did not mean 'not present' because the mouse bioassay system was insensitive to BSE infectivity. The classification indicated that there was no evidence that the tissues could propagate the disease.

In November 2003, at the request of the Chief Medical Officer for England, the Spongiform Encephalopathy Advisory Committee (SEAC) considered evidence on the potential transmission of vCJD from mother to child in utero and from breast milk. The committee had limited epidemiological and experimental research on the maternal transmission of prion diseases to work with. Using unpublished surveillance data on children born to vCJD mothers from the National CJD Surveillance Unit and the surveillance of progressive intellectual and neurological deterioration of neurological illness in children in the UK (Devereux and others 2004), the committee concluded that there was no epidemiological evidence for the maternal transmission of vCJD, including transmission by breast milk, but acknowledged that there was a hypothetical risk. The evidence was limited and mostly indirect but the risk appeared to be low for this high-risk group. The government position remains that infected milk should be banned from human consumption (SEAC 2004). Research in the UK, funded by the Department for Environment, Food and Rural Affairs and the Food Standards Agency concerning the safety of milk and the transmission of prions is continuing.

Despite the low risk determined from several research studies, one study pointed out the possibility of passing prions in milk. A Japanese woman dying of CJD was found to have the infectious prions in her colostrum (Tamai and others 1992). However, this result was later retracted because the Japanese research authorities that repeated the work found that lateral transmission could not be discounted as the route of exposure, and the results of the infectivity in milk were not repeatable. The secondary contamination of milk can virtually be excluded because milk from animals in commercial dairy operations is unlikely to contain colostrum (Vetrugno 2004).

Perhaps the most compelling evidence that milk constitutes a negligible risk comes from work by Everest and others (2006), who found that no abnormal prion proteins were detectable in the cell fraction of milk from cattle incubating BSE either by a modified commercial BSE ELISA or a second confirmatory assay.

Although clinically healthy animals do not appear to transmit prions, recent work by Ligios and others (2005) and Aguzzi (2006) suggests that inflammation in the secretory organs and the coexistence of a prion infection may lead to the contamination of secretions by prions. Concurrent diseases such as a viral infection of the mammary gland and inflammation may induce conditions in which prions are secreted in milk. Although the available evidence is limited, the health risk to human beings as a result of consuming milk or milk products from BSE-infected cows appears to be negligible.

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別紙様式第 2-1

別紙 3-5

# 医薬品
# 医薬部外品　研究報告　調査報告書
# 化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 12 月 8 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
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| 一般的名称 | | | 研究報告の公表状況 | Creutzfeldt-Jakob disease and blood transfusion: results of the UK transfu- sion medicine epidemiological review study. Hewitt, P.E. et al. Vox sanguinis, 91, 221-230 (2006) | 公表国<br>英国 | |
| 販売名（企業名） | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| この論文では，英国で実施中の大規模研究における 2006 年 3 月までの結果が報告された。本研究の目的は散発性 (sCJD)，家族性 (fCJD)，変異型クロイツフェルト・ヤコブ病 (vCJD) の輸血を介して伝播した証拠となるものがあるかどうかを明らかにすることである。vCJD18 例，sCJD3 例及び fCJD3 例の血液成分はそれぞれ 66，20，11 名の患者に輸血されたことが確認された。今日現在，vCJD 感染は 3 症例のみ確認されている。2 名の vCJD 感染者はそれぞれ白血球非除去赤血球製剤を輸血された 6.5 年 (R1)，7.8 年 (R2) 後に発症した。これらのドナーは献血後 40 ヵ月目と 21 ヵ月目にそれぞれ臨床症状を示していた。2 名の輸血受容者のプリオンタンパク遺伝子の 129 番目のコドンはホモ接合体のメチオニンであり，vCJD に感染しやすいことが確認されている。R1 は認知症と癌の併発で死亡 (後に vCJD と確認された)，その一方で R2 は「vCJD の可能性あり」と診断されたが 2006 年 3 月現在生存している。3 症例目 (R3) は献血の 18 カ月後に臨床症状を発症したドナーから赤血球の輸血を受けていた。R3 は輸血の 5 年後に CJD と無関係の原因で死亡，その 129 番目のコドンはヘテロ接合体であった。しかし，剖検の際，プロテアーゼ耐性の異常型 $PrP^{res}$ タンパクは，脾臓とリンパ節の一部で検出されたが，脳からは検出されなかった。そのため，この症例は発症前または無症候感染であったと考えられる。一方，fCJD 及び sCJD の輸血感染報告はなかったが，fCJD 及び sCJD 感染ドナーの症例数が少ないことを考慮すると，結論付けられる結果ではない。結論として，この継続中の研究において，おそらく過小評価していた輸血を介したヒト間での vCJD 感染リスクが存在することが明らかになった。 | BYL-2007-0263 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 英国で実施されたこの大規模な調査は，sCJD および fCJD については結論が出されていないが，既に懸念されている輸血を介した vCJD 感染の可能性については確認できたと考えられる。しかしながら，弊社の血漿分画製剤，コージネイト FS あるいはコージネイト FS バイオセット注の製造工程で使用されている血漿分画製剤は，米国で採集されているため，vCJD リスクは非常に小さいと考えられる。さらに，これら血漿分画製剤の製造工程では，非常に効果的であることが実験上確認されているプリオン除去工程が組み込まれている。 | 現時点で新たな安全対策上の措置を講じる必要はないと考える。 |



57

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ORIGINAL PAPER

# Creutzfeldt–Jakob disease and blood transfusion: results of the UK Transfusion Medicine Epidemiological Review study

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Vox Sanguinis

**Background and Objectives** This paper reports the results to 1 March 2006 of an ongoing UK study, the Transfusion Medicine Epidemiological Review (TMER), by the National CJD Surveillance Unit (NCJDSU) and the UK Blood Services (UKBS) to determine whether there is any evidence that Creutzfeldt–Jakob disease (CJD), including sporadic CJD (sCJD), familial CJD (fCJD), and variant CJD (vCJD) is transmissible via blood transfusion.

**Materials and Methods** Sporadic CJD and fCJD cases with a history of blood donation or transfusion are notified to UKBS. All vCJD cases aged > 17 years are notified to UKBS on diagnosis. A search for donation records is instigated and the fate of all donations is identified by lookback. For cases with a history of blood transfusion, hospital and UKBS records are searched to identify blood donors. Details of identified recipients and donors are checked against the NCJDSU register to establish if there are any matches.

**Results** CJD cases with donation history: 18/31 vCJD, 3/93 sCJD, and 3/5 fCJD cases reported as blood donors were confirmed to have donated labile components transfused to 66, 20, and 11 recipients respectively. Two vCJD recipients have appeared on the NCJDSU register as confirmed and probable vCJD cases. The latter developed symptoms of vCJD 6.5 years and 7.8 years respectively after receiving non-leucodepleted red blood cells (RBCs) from two different donors who developed clinical symptoms approximately 40 and 21 months after donating. A third recipient, given RBC donated by a further vCJD case approximately 18 months before onset of clinical symptoms, had abnormal prion protein in lymphoid tissue at post-mortem (5-years post-transfusion) but had no clinical symptoms of vCJD. CJD cases with history of transfusion: Hospital records for 7/11 vCJD and 7/52 sCJD cases included a history of transfusion of labile blood components donated by 125 and 24 donors respectively. Two recipients who developed vCJD were linked to donors who had already appeared on the NCJDSU register as vCJD cases (see above). No further links were established.

**Conclusion** This study has identified three instances of probable transfusion transmission of vCJD infection, including two confirmed clinical cases and one pre- or sub-clinical infection. This study has not provided evidence, to date, of transmission of sCJD or fCJD by blood transfusion, but data on these forms of diseases are limited.

**Key words:** blood, CJD, familial, sporadic, transfusion variant.

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## Introduction

Until 2004, it was generally accepted that Creutzfeldt–Jakob disease (CJD) had not been transmitted by blood transfusion.

Preliminary findings from sheep studies indicate that bovine spongiform encephalopathy (BSE) and scrapie can be transmitted by blood transfusion [1,2]. It is vital to find out whether this also applies to human transmissible spongiform encephalopathies (TSEs) and, in particular, variant CJD (vCJD). The UK is the only country where a significant outbreak of vCJD has occurred and is in a unique position to study this question which has important implications for public health policy. The results reported in this paper are from a study which is being carried out, with ethical approval, to investigate whether or not there is any evidence for the transmission of any type of CJD (sporadic, familial and variant) by blood transfusion.

## Materials and methods

### CJD surveillance

A surveillance system for CJD, the National CJD Surveillance Unit (NCJDSU), was established in the UK in 1990 with the aim of identifying all cases of CJD in the UK. The methodology of this study has been described previously [3], but in brief involves referral of suspected cases to the Unit from targeted professional groups, including neurologists and neuropathologists, review of suspects by a neurologist from the Unit and review of investigation results and neuropathological material when available. Cases are classified according to standard diagnostic criteria [4,5]. Onset of clinical symptoms for vCJD cases are estimated to the nearest month by NCJDSU on the basis of available clinical information. Details of past medical history, including blood donation or transfusion, are obtained from the family of suspected cases. Following the identification of vCJD in 1996 a collaborative study, the Transfusion Medicine Epidemiological Review (TMER), was established between the NCJDSU and UK Blood Services (UKBS) to search for evidence of transfusion transmission of CJD. The study was granted ethical approval by the local Research Ethics Committee.

### Notification of CJD cases with a history of donation

Sporadic CJD (sCJD) and familial CJD (fCJD) cases with a history of blood donation are notified to UKBS retrospectively. For vCJD, all patients who are old enough to have donated blood (> 17 years of age) are notified to UKBS at diagnosis, whether or not there is a known history of blood donation. Upon receipt of notification from the NCJDSU, a search is made for donor records. Current computer databases and archived records (computerized and paper-based records where appropriate) based at individual blood centres are searched using name, date of birth, and previous addresses as identifiers. For CJD cases reported as blood donors, information on dates and places of donation is also used to help locate past donor records. Where donor records are found, all components produced and issued to hospitals are identified and their fate determined, as recorded in hospital blood transfusion laboratory records. Recipient details are then checked against the NCJDSU register to establish if there is a match between these individuals and patients who have developed CJD. Recipients details are also flagged with the Office for National Statistics (ONS) to establish date and certified cause of death.

### Plasma used for UK fractionation

Independent of this study, and for regulatory reasons, all plasma derived from donations made prior to a diagnosis of vCJD is notified to UK fractionators so that appropriate actions can be taken.

### Notification of CJD cases with a history of transfusion

Information provided by relatives of CJD cases with a previous transfusion history is also passed to UKBS, who then liaise with appropriate hospital blood transfusion laboratories. The laboratories identify whether transfusions took place at the time and place indicated, and, if so, identify the components transfused to the case. Details of donation numbers, component type and date transfused are passed back to the local blood centre and this information is used to identify the donors. Donor details are checked against the NCJDSU register and with ONS. For both donors and recipients, searches before 1980 are now impractical as most hospital records are no longer extant.

### Related public health measures

The Department of Health (England) set up a committee (the CJD Incidents Panel) in 2000 to advise health authorities on the management of incidents where patients may have been put at risk of CJD through medical procedures. Cases where a blood donor or blood recipient has later developed vCJD have been referred to the Incidents Panel for consideration and further actions have been recommended and implemented. These further actions are outside the scope of this study and are not reported in this paper.

## Results

### vCJD cases with history of blood donation

*Identification of donors*

A total of 150 vCJD cases (out of a total of 160 cases on the NCJDSU register) who were old enough to have been potential blood donors have been notified to UKBS as of 1 March

Table 1 Recipients of blood donated by variant Creutzfeldt–Jakob disease cases by year and blood component transfused ($n = 66$)

| Year of transfusion | Blood component transfused | Number of recipients |
|---|---|---|
| 1980–1984 | Whole blood | 1 |
| | Red blood cells | 1 |
| 1985–1989 | Red blood cells | 2 |
| 1990–1994 | Red blood cells | 9 |
| 1995–1999 | Whole blood | 1 |
| | Red blood cells | 15 |
| | Red blood cells – buffy coat depleted[a] | 2 |
| | Red blood cells – leucodepleted[b] | 2 |
| | Fresh frozen plasma | 3 |
| | Cryo-depleted plasma | 1 |
| | Cryoprecipitate | 1 |
| | Platelets (pooled) | 1 |
| 2000–2004 | Red blood cells – leucodepleted | 23 |
| | Fresh frozen plasma – leucodepleted | 2 |
| | Platelets (pooled, leucodepleted) | 2 |

[a]Red cells with buffy-coat (containing most of the platelets and white cells) removed by centrifugation and physical separation.
[b]Red cells leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$/unit according to UK guidelines [6].

2006. Of these, 31 of 150 (21%) were reported to have been blood donors at various times in the past, although there is variation in the details of available information and the confidence of families in donation history.

Donor records were found for 24 vCJD cases, comprising 20 reported by relatives as blood donors and four additional cases with no reported donation history. Of these, 18 vCJD cases (12% of the total eligible to donate blood) were confirmed to have donated labile blood components, with the number of components made and issued for use in UK hospitals ranging from 1 to 14 per donor. Six vCJD cases were registered as donors, but had not donated labile blood components. Two of these had never attended sessions, three were deferred (due to past medical history, low haemoglobin value and illness, respectively) and one case had donated plasma for fractionation only (made from a single donation from which the red cells were discarded).

The search for donor records was negative in 11 of 31 (35%) vCJD cases reported as putative donors (three of whom allegedly donated well before the onset of the BSE epidemic in the 1980s). The information provided in these negative cases was minimal, except in one case where relatives were confident that regular donations (up to 50) had been made in the years leading up to 1993. Despite extensive searches no records were found; moreover, blood collection sessions had never been made at the purported venue. No explanation has been found for the lack of records, although discrepancies in some of the details given suggest that the history was not as certain as initially thought.

### *Labile components issued to hospitals*

Sixty-six labile components originating from 18 donors were issued to UK hospitals over the period 1981–2004 and transfused to patients according to blood transfusion laboratory records. A further nine components issued between 1982 and 1996 could not be traced by the relevant hospital. Table 1 gives the number of recipients transfused by year and the type of blood component transfused. Fifty-six recipients (85%) received red cells or whole blood, seven (11%) were transfused with labile plasma components or derivatives and three (4%) received pooled platelets made according to UK specifications in which the buffy-coat preparation containing platelets from the implicated vCJD donor was pooled with buffy coats from three other donors and resuspended in plasma from one of the four donations. Nearly half of the red cell recipients received red cells that had been leucocyte-depleted by pre-storage filtration to $< 5 \times 10^6$ leucocytes per unit (in 99% of units with 95% statistical confidence according to UK guidelines [6]) after the introduction of universal leucocyte depletion of the UK blood supply in 1999.

### *Recipients of blood components*

Patient identifiers are available for 66 recipients who received blood from 18 different donors who went on to develop vCJD. None of the 66 recipients had themselves donated blood between receiving their transfusion and early 2004 when the UKBS implemented a policy of excluding all donors transfused in the UK since 1 January 1980. It is of note that 41 (62%) recipients were aged over 60 years at the time of transfusion and were not eligible to donate. All living recipients ($n = 26$) have been informed of their risk and advised not to donate blood, tissues or organs. Three instances of probable transfusion transmitted vCJD infection have occurred, including two confirmed clinical cases and one pre- or subclinical infection. Of these, two cases have died, and one is still alive (see succeeding discussion). Figures 1 and 2 show the survival period for dead (transfusion to death) and live recipients (transfusion to 1 March 2006) of vCJD components, respectively, according to the interval between transfusion and onset of clinical symptoms in the donor.

### *Dead recipients*

Forty recipients (61%) are known to be dead, with mean age at death 66 ± 19 years. Table 2 gives the time and cause of death as stated on death certificates for the recipients known to have died. Around half ($n = 21$) of the dead recipients died within a year of receiving their transfusion, with only seven surviving for more than 5 years. Two recipients, who died 4 months and 14 months, respectively, after transfusion had

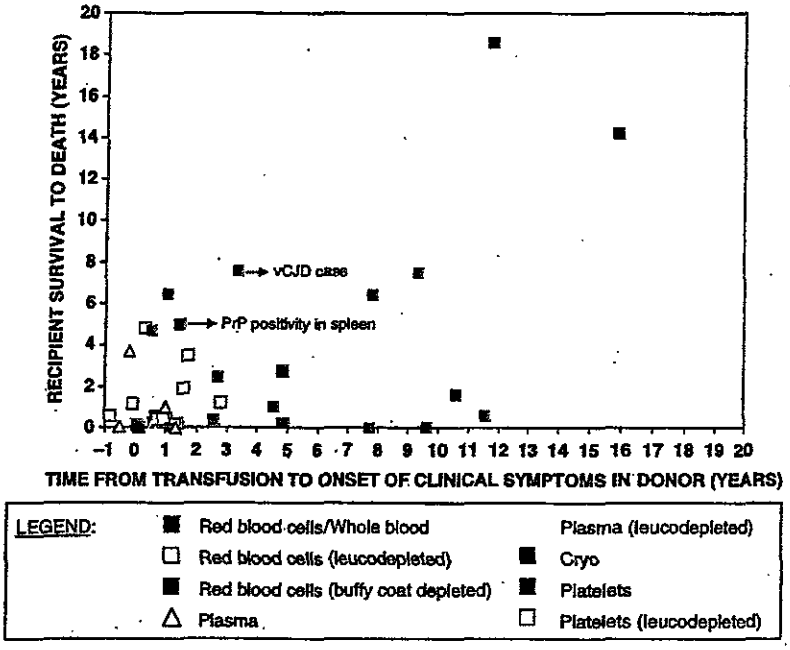


Fig. 1 Survival period (transfusion to death) for recipients of variant Creutzfeldt–Jakob disease components according to interval between transfusion and onset of clinical symptoms in the donor.

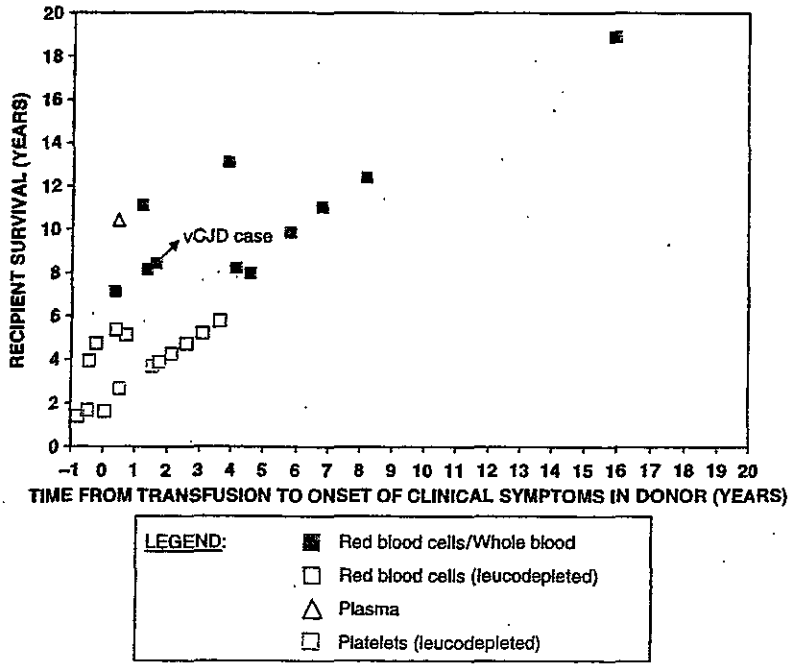


Fig. 2 Current survival period (since transfusion to 1 March 2006) for recipients of variant Creutzfeldt–Jakob disease components according to interval between transfusion and onset of clinical symptoms in the donor.

'dementia' recorded on the death certificate, but examination of case notes indicated that neither case had features to suggest vCJD. All the other recipients were certified as dying of causes unrelated to vCJD, except for a recipient whose cause of death on the death certificate was recorded as '1A dementia and II. prostate cancer' and was later confirmed neuropathologically as suffering from vCJD [7]. This patient, who had received a transfusion of red cells 6.5 years before onset of clinical symptoms, was a methionine homozygote at codon 129 of the human prion protein gene (*PRNP*). The case that donated to this individual also had a neuropathological diagnosis of vCJD, with clinical onset approximately 40 months after donating. In a second red cell recipient (of a different donor who developed clinical symptoms approximately

Table 2 Cause of death of variant Creutzfeldt–Jakob disease recipients known to have died ($n = 40$)

| Interval from transfusion to death | Number of recipients | Cause of death |
|---|---|---|
| < 1 month | 7 | Acute renal cortical necrosis |
| | | Cancer (2) |
| | | Myocardial infarction |
| | | Septicaemia (2) |
| | | Sepsis (pancreatitis) |
| 1– < 6 months | 11 | Aspiration pneumonia (sigmoid resection) |
| | | Cancer (4) |
| | | Myelodysplasia (2) |
| | | Myelofibrosis |
| | | Peritonitis (2) |
| | | Stroke/diabetes mellitus/dementia |
| 6– < 12 months | 3 | Cancer (3) |
| 1– < 5 years | 12 | Acute myeloid leukaemia (2) |
| | | Bronchopneumonia/senile dementia |
| | | Cancer |
| | | Ischaemic heart disease (3) |
| | | Chronic obstructive airways disease (COAD) |
| | | Hypertensive heart disease, chronic renal failure |
| | | Myelodysplasia |
| | | Disseminated sepsis |
| | | Spinal haemangioblastoma |
| 5– < 10 years | 5 | Cerebrovascular accident |
| | | Ischaemic heart disease |
| | | Acute lymphoblastic leukaemia |
| | | Dementia[a], prostatic cancer |
| | | Ruptured aortic abdominal aneurysm/severe atheroma/COAD[b] |
| ≥ 10 years | 2 | Bronchopneumonia |
| | | Ischaemic heart disease |

[a]Confirmed variant Creutzfeldt–Jakob disease case [7].
[b]PrP positivity in lymphoid tissue, pre- or subclinical vCJD infection [8].

18 months after donating and was later diagnosed with neuropathologically confirmed vCJD), protease-resistant prion protein ($PrP^{res}$) was detected in the spleen and one lymph node (but not in the brain) at post-mortem [8]. This recipient, who died 5 years after transfusion without any clinical symptoms of vCJD, was a codon 129 *PRNP* heterozygote and is thought to represent pre- or subclinical infection.

### *Live recipients*

Twenty-six recipients (39%) are alive as of 1 March 2006 with a mean age of 63 ± 19 years. Table 3 shows the number of live recipients according to the time elapsed since transfusion, along with their current age, component transfused and the interval between donation and onset of clinical symptoms of vCJD in the donor. Fifty per cent of live recipients were transfused with components from vCJD donors whose donations were made within 20 months of clinical onset, in seven cases around the time of development ($n = 3$) or shortly after ($n = 4$) the first signs of clinical illness. These cases would have appeared healthy when attending donor sessions and passed the normal medical checks as being fit to donate. Sixteen recipients have survived longer than 5 years, with six surviving > 10 years (one for over 18 years). These patients, mean age currently 61 ± 19 years, were given blood from donors who developed vCJD symptoms at intervals ranging from around 5 months to 191 months after making the donation (see Table 3). Recently, a diagnosis of probable vCJD has been made in one of these surviving recipients who had received a transfusion of red cells 7 years and 10 months before onset of clinical symptoms [9]. The donor of this third probable transfusion-transmitted vCJD infection developed vCJD approximately 21 months after the donation, and the recipient is a codon 129 *PRNP* methionine homozygote.

### *Plasma for UK fractionation*

Twenty-five units of plasma originating from 11 different donors, bled between 6 months and 17 years, 11 months before onset of clinical vCJD symptoms, were supplied for UK fractionation during the period 1986–1998. Product batches manufactured from 23 plasma units derived from nine donors have been traced. The fate of batches of product derived from the two remaining plasma donations, from two different donors, has not yet been traced, and this search is still ongoing. Table 4 lists the plasma products derived from the 23 traced donations and the number of batches implicated, divided into risk categories as used in the plasma product notification exercise (www.hpa.org.uk/infections/topics_az/cjd/Recommendations.pdf). The fate of batches of products has not been traced to individual recipients as part of this study. It is known, however, that haemophilia centres have traced the ultimate fate of the batches of factor VIII. It is also known that no case of vCJD has been identified in a patient with haemophilia in the UK.

## sCJD cases with history of blood donation

Ninety-three cases of sCJD identified between 1980 and 2000 were reported to have been blood donors, with only 38 reported to have donated from 1980 onwards. Donation records for most sCJD cases were untraceable since most dated back many years before 1980, in some cases to the 1940s. Donation records were found for eight sCJD cases, but only three had actually donated labile blood components for hospital use (one with 18 recipients, and one each with one recipient) which could be traced to recipients. A total of 20 recipients were transfused between 1995 and 1999 with components from these three donors who went on to develop

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Table 3 Live recipients of labile blood components donated by variant Creutzfeldt–Jakob disease cases ($n = 26$)

| Time elapsed since transfusion[a] | Current age of recipient (years)[a] | Blood component transfused | Interval between blood donation and onset of clinical symptoms in donor (months)[b] |
|---|---|---|---|
| 1 – < 2 years | 48 | Platelets (leucodepleted) | –9 months |
| | 49 | Red cells (leucodepleted) | –5 months |
| | 83 | Red cells (leucodepleted) | 2 months |
| 2 – < 3 years | 38 | Red cells (leucodepleted) | 7 months |
| 3 – < 4 years | 58 | Red cells (leucodepleted) | 19 months |
| | 83 | Red cells (leucodepleted) | 22 months |
| | 90 | Red cells (leucodepleted) | –5 months |
| 4 – < 5 years | 59 | Red cells (leucodepleted) | 26 months |
| | 67 | Red cells (leucodepleted) | –2 months |
| | 89 | Red cells (leucodepleted) | 32 months |
| 5 – < 6 years | 30 | Red cells (leucodepleted) | 37 months |
| | 52 | Red cells (leucodepleted) | 9 months |
| | 64 | Red cells (leucodepleted) | 44 months |
| | 71 | Red cells (leucodepleted) | 5 months |
| 6 – < 7 years | – | – | – |
| 7 – < 8 years | 42 | Red cells | 5 months |
| 8 – < 9 years | 31[c] | Red cells | 21 months |
| | 74 | Red cells | 17 months |
| | 76 | Red cells | 49 months |
| | 87 | Red cells | 55 months |
| 9 – < 10 years | 75 | Red cells | 70 months |
| > 10 years | 33 | Cryo-depleted plasma | 7 months |
| | 49 | Red cells | 15 months |
| | 67 | Red cells | 46 months |
| | 70 | Red cells | 191 months |
| | 75 | Red cells | 98 months |
| | 87 | Red cells | 82 months |

[a]As at 1 March 2006.

[b]A negative interval denotes that donation was made by individual while (retrospectively recognized) clinical symptoms were present.

[c]Probable variant Creutzfeldt–Jakob disease case [9].

sCJD between 1 and 5 years after donation. Of these, 11 (55%) received red cell components, eight recipients (40%) received platelets and one (5%) received fresh frozen plasma.

As of 1 March 2006, 12 recipients are confirmed dead with a mean age at death of 74 ± 15 years. Of these, five died soon after transfusion (four within a week, and one 2 months later) and seven survived for between 1 and 8 years after receiving their transfusion before dying of a variety of non-CJD-related causes (cerebrovascular accident/stroke, $n = 3$; acute myeloid leukaemia, $n = 3$, general debility/old age, $n = 1$). Seven recipients are not known to be dead from ONS flagging to date, and are therefore presumed to be alive. The mean age of these seven recipients is 58 ± 19 years. The time elapsed since their transfusion ranges from 7 to 9 years. The fate of a further recipient is unknown. None of the sCJD recipients identified as having received blood from donors who went on to develop sCJD have appeared on the NCJDSU register to date.

## fCJD cases with history of blood donation

Donation records were found for three out of five cases of fCJD identified between 1992 and 2000, all reported to have donated blood after 1980. These three cases had all donated labile blood components (one with five recipients, one with four recipients and one with two recipients) for hospital use which could be traced to individual recipients. A total of 11 recipients were transfused between 1977 and 1992 with labile components from these three donors who went on to develop fCJD between 1 and 15 years later. Nine of the 11 (82%) recipients received red cell components (whole blood $n = 6$, red cells $n = 3$) while two received platelets.

Five of 11 recipients identified have since died with a mean age at death of 75 ± 6 years. Three of these survived for 3, 10 and 17 years after transfusion before dying of non-CJD-related causes (cancer, $n = 2$; bronchopneumonia, $n = 1$); and two died of cancer shortly after receiving their transfusion.

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Table 4 Product batches made by UK fractionators derived from plasma donated by individuals who later developed variant Creutzfeldt–Jakob disease[a,b]

| Infectivity Classification[c] | Plasma product | Number of implicated batches |
|---|---|---|
| Low | Factor VIII (excipient[d]) | 77 |
| | Albumin 20% | 21 |
| | i.m. immunoglobulin | 12 |
| Medium | Albumin 4-5% | 28 |
| | i.v. immunoglobulin | 11 |
| High | Factor VIII | 16 |
| | Factor IX | 8 |
| | Anti-thrombin | 1 |
| | TOTAL | 174 |

[a]Twenty-three plasma donations from nine variant Creutzfeldt–Jakob disease donors, data courtesy of Health Protection Agency.
[b]Excludes fate of two plasma units from two further vCJD cases (see text for explanation).
[c]Risk categories as used in plasma product notification exercise.
[d]Albumin from implicated plasma donation used as excipient (inert substance added to provide bulk) in preparation of batch of Factor VIII.

Three recipients are not known to be dead from ONS flagging to date, and are therefore presumed to be alive. The mean age of these three recipients is 44 ± 20 years. The time elapsed since their transfusion ranges from 13 to 21 years. The fate of a further three recipients is not known. None of the fCJD recipients identified as having received blood from donors who went on to develop fCJD have appeared on the NCJDSU register to date.

### vCJD cases with history of transfusion

Eleven vCJD cases were reported to have received past blood transfusions between 1962 and 1999. A further case received a blood transfusion after onset of illness. This case is excluded from further analysis. For two cases, hospital records showed that they had not been transfused. No hospital records could be found for another two cases reported to have been transfused in 1962 and 1971, respectively. Hospital transfusion records were found for seven vCJD cases (64% of those reported as transfused) who had been transfused with components donated by 125 donors (121 identified), with one vCJD case, who also received a solid organ transplant, receiving components from 103 donors. The identity of four donors who donated red cell/whole blood components to two cases (case 2 and case 7, see Table 5) is unknown. Table 5 shows the transfusion date, number of donors and blood components donated, and the interval from transfusion to onset of clinical symptoms of vCJD in these seven recipients. These cases had been exposed to between two and 103 donors, respectively (NB search for donors to case 6 is incomplete). To date, one donor who gave red cells to case 5 and another donor who gave red cells to case 6 are also registered on the NCJDSU database as vCJD cases. These are the donors of the two clinical cases of transfusion-transmitted vCJD referred to previously (see vCJD cases with history of donation).

### sCJD cases with history of transfusion

Fifty-two cases of sCJD identified between 1980 and 2000 were reported to have received a blood transfusion, of which 28 received a transfusion after 1980. Transfusion records were found for seven sCJD cases transfused between 1984 and 1997. Donor details were found for 24 donors who donated components transfused to these seven sCJD cases. One of these donors is known to have died, with a cause of death not related to CJD. Twenty donors are not known to have died from ONS flagging to date, and are therefore presumed to be alive. The fate of a further three donors is not known. The mean age of the donors presumed still alive is 51 ± 9 years. None of the traced donors who gave blood to patients who were subsequently diagnosed with sCJD have appeared on the NCJDSU register to date.

### fCJD cases with history of transfusion

One case of fCJD identified in 1992 was reported to have received three blood transfusions in 1965, 1970, and 1987 none of which could be traced.

## Discussion

This study has identified three instances in which a recipient of a transfusion derived from a 'vCJD' donor has developed infection with vCJD, including two clinical cases and one pre- or subclinical infection [7–9]. These are three different donor/recipient pairs. In view of the small size of the total at-risk recipient population ($n = 66$) and the background mortality rate for vCJD in the general UK population (0·24/million/annum), these observations provide strong evidence that vCJD can be transmitted from person to person through blood transfusion. This finding has had important implications for public health policy nationally and internationally.

The risk of developing vCJD infection in the surviving recipient population is significant but cannot be precisely estimated because of variables including the timing of blood donation in relation to clinical onset in the donor, the influence of the codon 129 genotype of donor and recipient and the effect of the introduction of leucodepletion in 1999. Furthermore, the currently observed number of infections in the recipient population may be an underestimate as some surviving recipients may yet develop vCJD and there is limited available information on the outcome in the cohort of

Table 5 Donors ($n$ = 125) of labile blood components given to variant Creutzfeldt–Jakob disease cases[a] ($n$ = 7) with identifiable past hospital transfusion records

| Case | Transfusion date | Number of donors of labile blood components transfused | Blood component donated to vCJD recipient | Interval from transfusion to onset of illness |
|---|---|---|---|---|
| 1 | 1993 | 38 | Cryoprecipitate (4)<br>Fresh frozen plasma (11)<br>Platelets (8)<br>Red cells (14)<br>Whole blood (1) | 4 years, 9 months |
| 1 | 1993 | 65 | Cryoprecipitate (12)<br>Fresh frozen plasma (25)<br>Platelets (17)<br>Red cells (11) | 4 years, 6 months |
| 2 | 1983 | 2[b] | Red cells | 15 years, 11 months |
| 2 | 1993 | 3 | Fresh frozen plasma | 6 years, 3 months |
| 3 | 1994 | 4 | Red blood cells | 5 years, 4 months |
| 4[c] | 1999 | 5 | Red blood cells (2)<br>Red blood cells (Leucocyte-depleted) (3) | 8 months |
| 5[a] | 1996 | 5[d] | Red blood cells | 6 years, 6 months |
| 6[a] | 1997 | 1+[e] | Red blood cells | 7 years, 10 months |
| 7 | 1982 | 2[b] | Whole blood | 13 years, 11 months |

[a]Two of these cases linked to donors already on the National CJD Surveillance Unit (NCJDSU) register as vCJD cases [7,9].
[b]Component details traced, but donors not identifiable.
[c]Timing of clinical illness excludes blood transfusion as the source of infection in this case.
[d]One of the donors already on NCJDSU register as vCJD case, others presumed not to be source of infection.
[e]One donor already on NCJDSU register as vCJD case. Search for 40+ donors to Case 6 not complete, as of 1 March 2006.

deceased recipients; a significant proportion of these individuals may not have survived long enough to express clinical disease even if infected. The minimum incubation period in CJD transmitted from person to person by a peripheral route is 4·5 years in kuru and growth-hormone-related CJD [10,11] and only nine deceased recipients survived for longer than this period. An investigation of the hospital records of the deceased recipients is underway, and to date, none had clinical features of vCJD pre-mortem. However, the identification of the individual with 'preclinical' vCJD infection was dependent on post-mortem examination of peripheral lymphoreticular tissues, and, to date, no equivalent tissues have been available in the deceased transfusion recipients. Extrapolating from the three observed infections in the total recipient population is likely to lead to an underestimate of the overall risk of transfusion transmission of vCJD, although the introduction of leucodepletion in 1999 may have reduced the risk to recipients transfused after this date.

A further important variable in estimating individual risk is the time from blood donation to clinical onset in the donor and, although evidence from animal studies in relation to this issue is conflicting [12–14], it is likely that an extended gap between blood donation and clinical onset in the donor will reduce the risk of transfusion transmission. All tested clinical cases of vCJD have been methionine homozygotes at codon 129 of *PRNP*, but the individual with 'pre-clinical' transfusion transmitted infection was heterozygous at this locus [8], indicating that individuals with this genotype are susceptible to secondary infection with vCJD. Except for the three cases infected through blood transfusion, the codon 129 genotypes of the recipient population are not known. Although the relative risk of secondary infection in relation to the codon 129 genotype is uncertain, a recent study in a transgenic mouse model suggests that individuals with all human codon 129 genotypes may be susceptible to secondary infection with vCJD, with a hierarchy of risk from methionine homozygotes to heterozygotes to valine homozygotes [15]. Risk may vary according to genetic background, but it cannot be assumed that some recipients will possess an absolute genetic barrier to infection.

The analysis of vCJD cases with a history of blood transfusion has identified over 100 donors to these cases, although the great majority were linked to one vCJD case who had undergone an organ transplant. A risk assessment has suggested that these donors are themselves at significant risk of developing vCJD and these individuals have been informed of this risk and have been advized not to act as blood or organ donors. To date, none of these individuals have developed vCJD, with the exception of the two donors linked to the two clinical cases of vCJD described above.

Plasma derived from vCJD cases has been used in the production of plasma derived products, including clotting factors and immunoglobulin. To date, there is no evidence that vCJD has developed in a recipient of these products. However, the potential incubation period from a presumed low dose exposure by a peripheral route may be prolonged and current observational data cannot exclude the possibility of transmission of vCJD through plasma products. A risk assessment carried out in the UK suggested that, on worst case assumptions, some plasma products could be associated with an additional risk of developing vCJD in relation to the background population risk through exposure to BSE and since 1999 plasma for the production of plasma products has been imported to the UK from other countries. In contrast to labile blood components, plasma products are manufactured using a production process, some steps of which may reduce TSE infectivity [16]. Together with the estimated relatively low levels of initial infectivity in plasma used in fractionation, the risks from plasma products are probably much lower than the risks from transfusion of labile blood components.

An important question raised by the evidence of transfusion transmission of vCJD is whether other human TSEs may be transmissible through this mechanism. Cumulative evidence in sCJD over many years does not suggest that sCJD is transfusion transmitted [17]. Case-control studies have not demonstrated an increased risk of sCJD through a past history of blood transfusion [18–20] and lookback studies have not linked blood transfusions derived from sCJD blood donors to sCJD cases [21,22]. However, the case-control methodology may be compromised by control selection (some studies of CJD used hospital based controls) and this type of research cannot exclude rare transfusion transmission events. The lookback study of sCJD (and fCJD) from the TMER study provides only limited evidence, not least because of the difficulties in tracing blood donations made years or decades in the past, and there is only one study of sCJD equivalent to the TMER study of vCJD [22]. This has not provided evidence of transfusion transmission of sCJD, despite prolonged periods of follow-up in a proportion of cases. There is however a need to continue the study of blood transfusion and sCJD, and this will become of particular importance should highly sensitive tests for infectivity in blood be developed [23]. There is recent evidence of prion protein deposition in peripheral tissues in sCJD, including muscle [24]. However, the increased lymphoreticular involvement in vCJD in comparison to sCJD may be associated with an increased risk of transfusion transmission of vCJD.

The TMER study has provided compelling evidence that vCJD is transmissible through blood transfusion, representing the first evidence of transmission of human TSEs through this route and via material sourced from a peripheral tissue rather than the high level infectivity tissues of the central nervous system. The manifest implications for public health of transmission transfusion of vCJD have led to measures to minimize the risk from blood transfusion [25] and plasma products derived from cases incubating vCJD and many of these actions were taken years in advance of the evidence for transfusion transmission, both in the UK and many other countries. Although there is uncertainty about the potential for transfusion transmission of vCJD to lead to a self-sustaining epidemic, the introduction of a policy of deferring transfusion recipients as blood donors in the UK has minimized this possibility. Other actions to reduce the risk, such as the introduction of filtration devices, are under consideration in the UK, but the identification of vCJD cases with a history of blood donation in France, Ireland, Spain and Saudi Arabia indicates that this issue has an international dimension. One determinant of the risk is the population prevalence of vCJD infection, which is almost certainly highest in the UK, but the identification of secondary transmission of vCJD underlines the importance of international surveillance systems both for human and animal prion diseases.

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| 一般的名称 | | | 研究報告の公表状況 | Variability of parvovirus B19 to inactivation by liquid heating in plasma products<br>Hattori, S. et al.,<br>Vox Sanguinis, 92, 121-124 (2007) | 公表国<br>日本 | |
| 販売名（企業名） | | | | | | |

| 研究報告の概要 | 使用上の注意記載状況・その他参考事項等 |
|---|---|
| 過去の研究から，ヒトパルボウイルス B19 は液体-熱不活化に対して，溶液の組成に大きく依存する複雑な感受性を示すことが明らかとなっている。また，これらの研究では，ヒトパルボウイルス B19 のモデルとして一般的に用いられる動物パルボウイルスは必ずしも適切ではないという事実を強調している。本稿では液体-熱処理時の，ハプトグロビン中及び抗トロンビン製剤中のヒトパルボウイルス B19 の感受性を明らかにしている。60℃にてさまざまな培養時間で培養後，各試料中に残存する感染性を，細胞ベースアッセイによって測定した。その結果，ハプトグロビン溶液中で緩徐ではあるが実質的（最大では完全）な不活化が可能であったが，一方，抗トロンビン溶液中では限定的な不活化しか確認できなかった。しかしながら，この結果は，多くのパラメータ（pH 値，クエン酸塩濃度及び塩化ナトリウム，ならびにそれぞれの溶液で用いられる安定剤）がヒトパルボウイルス B19 の熱感受性に影響することを考慮すると単純なものではないと考えられる。したがって，著者らはそれぞれの実験条件の下，慎重に評価することを提言している。 | BYL-2007-0277 |

| 報告企業の意見 | 今後の対応 |
|---|---|
| 弊社の血漿分画製剤に使用されるミニプール血漿においては，ヒトパルボウイルス B19 に対する NAT を実施しており，10E5 IU/mL 以上が確認された場合は，そのミニプール血漿は製造工程から除去している。現在の科学水準では，ヒトパルボウイルス B19 を確実に不活化する方法は存在しないため，感染リスクを完全に排除することはできないと考える。 | 今回，得られた情報は感染リスクの変化を示唆するものではないことから，現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き情報収集に努める。 |



56

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ORIGINAL PAPER

# Variability of parvovirus B19 to inactivation by liquid heating in plasma products

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Vox Sanguinis

**Background and Objectives** Previously, we reported that although human parvovirus B19 in albumin and intravenous immunoglobulin preparations was rapidly inactivated during liquid heating, in contrast to other parvoviruses such as canine parvovirus, sensitivity to heat was highly dependent on the composition of the solution. In this study, we aimed to further elucidate the sensitivity to heat of B19 in haptoglobin and antithrombin (previously named antithrombin III) preparations during liquid heating.

**Materials and Methods** Two different solutions collected immediately before heat treatment of haptoglobin and antithrombin preparations were spiked with B19 and subsequently treated at 60 °C for 10 h. B19 DNA-positive, anti-B19 IgG/IgM-negative plasma was used as a source of B19. The residual infectivity in each sample was measured using a B19 cell-based infectivity assay with an mRNA polymerase chain reaction.

**Results** B19 in different plasma preparations showed different heat-sensitivity patterns during liquid heating: (i) slow inactivation in haptoglobin preparations, and (ii) only limited inactivation in antithrombin preparations. The kinetics of inactivation was greatly different from that in our previous studies in which the virus was shown to be rapidly inactivated in albumin and intravenous immunoglobulin preparations.

**Conclusion** B19 has unique properties in terms of heat sensitivity, depending on the composition of the solution during liquid heating. This finding may indicate the need for caution when interpreting the sensitivity of B19 to heat.

**Key words:** heat sensitivity, parvovirus B19, plasma products, viral inactivation, viral safety.

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## Introduction

Human parvovirus B19 (B19) is not highly pathogenic to healthy individuals; however, this virus does present a certain level of risk, because it may cause serious pathological conditions in pregnant women and immunosuppressed individuals. As B19 has no envelope, it is resistant to detergent or organic solvent treatments. In addition, B19 is a small virus (18–26 nm in diameter), which means that it is not easy to eliminate by filtration. Liquid-heat treatment, i.e. pasteurization, considered a highly reliable method of inactivating viruses, has been introduced into the manufacturing process for the production of various plasma derivatives. Investigations using model animal parvoviruses suggested that viruses like B19 are heat resistant, indicating that the potential for inactivation of this virus in plasma products may be limited [1]. In addition, the European Agency for the Evaluation of Medical Products and Japanese Ministry of Health, Labor and Welfare have issued guidance for risk evaluation of non-enveloped viruses in plasma products [2,3].

Interestingly, recent reports have shown that under liquid-heating conditions in albumin preparations and heat-treated/

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PEG-treated intravenous immunoglobulin (IVIG) preparations, B19, unlike other animal parvoviruses such as canine parvovirus (CPV) and porcine parvovirus (PPV), was highly heat sensitive and was rapidly inactivated [4–6]. Unexpectedly, however, during heat treatment, the B19 in a sucrose solution was not inactivated rapidly [5]. In addition, B19 was also resistant to inactivation by dry heating [5]. Therefore, in this study, we aimed to elucidate further the sensitivity to heat of B19 in haptoglobin and antithrombin (previously named antithrombin III) preparations by liquid heating.

## Materials and methods

### Test materials

Plasma collected according to the regulation in the USA, and negative for anti-human immunodeficiency virus (HIV) 1/2 IgG, hepatitis B virus (HBV) surface antigen, anti-hepatitis C virus (HCV) IgG, and negative for HIV RNA, HBV DNA and HCV RNA additionally, but positive for B19 DNA (negative for anti-B19 IgG), was used (Plasma ID: P4). This plasma sample was the same as the one used previously for an evaluation of heat sensitivity in albumin and IVIG preparations [5,6]. Two different solutions collected immediately before heat treatment of haptoglobin and antithrombin preparations were used (Haptoglobin Injection-Yoshitomi and Neuart®, respectively; Benesis Corporation, Osaka, Japan).

### B19 infectivity assay

The infectivity of B19 in the samples was titrated using KU812 cells (JCRB0104; Health Science Research Resources Bank, Tokyo, Japan) and subsequently viral mRNA in positive wells was detected with reverse transcriptase–polymerase chain reaction (RT-PCR) systems. Briefly, the KU812 cells were inoculated with 10-fold serial dilutions of B19 and cultured in RPMI-1640 medium containing 10% fetal bovine serum, 6 IU/ml erythropoietin (Sankyo Co., Ltd, Tokyo, Japan), 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen Corp., Carlsbad, USA), 1 mM sodium pyruvate (Invitrogen Corp.), and an ITS-X supplement (Invitrogen Corp.) for 4 days [5]. Wells were monitored for the presence or absence of viral mRNA, and the viral titre remaining after heat treatment was calculated according to the Kärber method [7]. We used two different PCR systems. For the experiment using haptoglobin preparations, RNA was extracted and viral mRNA was amplified as described previously [5]. For the experiment using antithrombin preparations, total RNA was extracted from the infected cells (ABI PRISM 6100 system; Applied Biosystems, Foster City, CA, USA) and employed for amplification of the B19 VP2 exon region with the sense primer B19-21 5′-TGGCAGACCAG-TTTCGTGAA-3′ (nt 2162–2181), antisense primer B19-22 5′-CCGGCAAACTTCCTTGAAAA-3′ (nt 3086–3067) and probe B19-F23 5′-FAM-CAGCTGCCCCTGTGGCCC-3′ (nt 3048–3065), using a TaqMan One-step RT-PCR Master Mix Reagents Kit and ABI 7500 Real-Time PCR system (Applied Biosystems) [8]. These primers and the probe were optimized for the ABI system based on previous designs.

### Porcine parvovirus infectivity assay

ST cells (porcine testis cell line; ATCC CRL-1746) were inoculated with 10-fold serial dilutions of the NADL-2 strain of PPV (ATCC VR-742) and cultured in minimum essential medium supplemented with 10% fetal calf serum for 10 days. Wells were monitored for the presence or absence of viral cytopathic effects, and the viral titre remaining after heat treatment was calculated according to the Kärber method [7].

### Heat treatment of B19 and porcine parvovirus

Heat treatment of B19/PPV was performed as described previously [5]. Briefly, the B19-positive plasma sample was diluted with 10 volumes of PBS. The diluted B19-positive plasma was precipitated by ultracentrifugation at 150 000 *g* for 3 h and the resulting particulate fraction was resuspended in 2 volumes using the intermediate product. The samples containing B19 were then aliquotted and incubated in a water bath at 60 °C, or 37 °C for the control, for preset periods of time. After immediate cooling, the remaining infectivity titre of B19 was measured as described above. PPV experiments were also performed in the same way.

## Results

To evaluate the ability of heat treatment to inactivate B19 in the product's intermediate, the virus was inoculated into two different samples. After being heated at 60 °C for 10 h, the samples for kinetic experiments on the heat inactivation of B19 were taken at preset time points. The B19 was inactivated by 6·3 logs and 2·1 logs after 10 h by liquid heating of haptoglobin and antithrombin preparations, respectively, whereas PPV was inactivated by 3·2 logs and 2·1 logs, respectively. No inactivation of B19 was observed on heating at 37 °C for 10 h in the control (data not shown). The kinetics of inactivation in the haptoglobin preparations differed between B19 and PPV, i.e. the infectivity of B19 was reduced near detection limit at 5 h, whereas the infectivity of PPV remained even at 10 h (Fig. 1). On the other hand, the kinetics of inactivation in the antithrombin preparations was comparable and the levels of infectivity remaining even after inactivation for 10 h were similar (Fig. 1).

## Discussion

We previously reported that B19 was inactivated rapidly in albumin and IVIG preparations during liquid heating at

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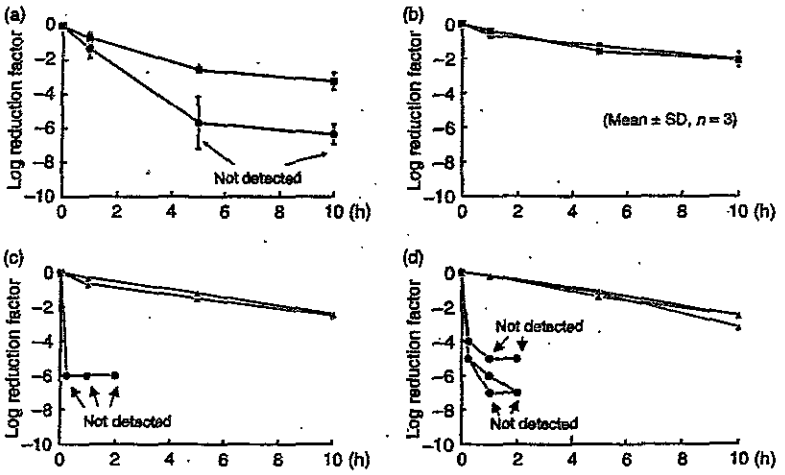


**Fig. 1** Heat inactivation kinetics of parvovirus B19 in several plasma products. B19 was introduced into plasma samples, that were collected immediately before heat treatment of haptoglobin (a) and antithrombin (b) preparations. The samples containing B19 were then aliquotted and incubated in a water bath at 60 °C, or 37 °C for the control, for preset periods of time (0, 1, 5, and 10 h). The infectivity of B19 remaining after heating (●) was measured. In the haptoglobin preparations, no infectivity was detected in two of three independent experiments after 5 and 10 h of heat treatment. Porcine parvovirus (■) was also evaluated for comparison. The inactivation kinetics of B19 and CPV (▲) in albumin and intravenous immunoglobulin preparations are shown in (c) and (d), respectively. The results in (c) and (d) are derived from references [5,6], respectively, with minor modifications.

60 °C, whereas B19 in a sucrose solution was not inactivated quickly [5]. In the present study, we found that the heat sensitivity of B19 in haptoglobin as well as antithrombin preparations during liquid heating at 60 °C was substantially different from previous results determined under equivalent heating conditions with albumin and IVIG preparations. Thus, B19 showed different inactivation kinetics during liquid heating, depending on the product: (i) rapid inactivation within a few hours; (ii) slow inactivation that takes 10 h; and (iii) little inactivation even after heating for 10 h (i.e. comparable with the inactivation kinetics obtained with CPV or PPV as a model virus). Liquid heating of these samples was performed with the stabilizers used in the preparation of the products. Such stabilizers had been selected based on their abilities to stabilize plasma proteins and to inactivate viruses. In this study, we used a haptoglobin preparation containing glycine and an antithrombin (AT) preparation containing sodium chloride/trisodium citrate dihydrate, as stabilizers, as described in the Materials and methods. On the other hand, liquid heating for the albumin and IVIG preparations reported in our previous papers was performed with sodium caprylic acid/acetyl tryptophan and D-sorbitol, respectively, as stabilizers. Thus, we could not identify which component of the stabilizers for the haptoglobin and antithrombin preparations plays a role in the reduction in B19's heat sensitivity, because there is no component included in the stabilizers for these preparations but not in those for the albumin and IVIG preparations. Concentration of citrate during heat treatment was generally 0·5–1·0 M [9]. Our manufacturing condition was located in this range. However, our preliminary experiments suggest that the B19 heat sensitivity was influenced by several constituents such as concentration of citrate, sodium, chloride, and pH, during AT heat treatment (data not shown). This finding suggests that the parameter of B19 stabilizing during heat treatment is not so simple and it seems to be complex. Thus, B19 heat sensitivity during heat treatment should be evaluated in each process conditions avoiding an over estimation for the B19 inactivation. We are now trying to identify the components of the stabilizers that contribute to the decrease in the sensitivity of B19 to heat.

Umemori *et al.* [10] presented that even after heat treatment at 60 °C for 10 h, a B19-containing albumin preparation remained infective as assayed using Niigata embryonal carcinoma (NEC) and KU812 cells. However, comparable results, i.e. inactivation to near the limits of detection [10], or below the limit of detection in our previous study [5], were also obtained in the present study. This slight difference may be derived from the use of a cold spot using heat-block system [10] vs. complete sinking into a water bath [5,6]. The sensitivity of B19 to heat was also shown to be influenced by the residual moisture in the sample preparations during dry heating [5,11]. These findings strongly suggest that the sensitivity of B19 is highly dependent on the conditions during heating. Therefore, it is necessary to design a study for the evaluation of safety with respect to contamination by and/or elimination of B19 during the manufacturing process, especially when heating steps are investigated.

Canine parvovirus or PPV has been used at many facilities as a model for B19. We confirmed that these two viruses are useful for modelling B19 as they are more resistant to liquid heating than B19. Similarly, Prikhod'ko [12] also proposed that PPV is a useful model for B19 in dry heating. However, attention should be given to the sensitivity of B19 to heat which could be affected by the stabilizer used, as found in this study. We do not know the mechanism behind the clear difference in sensitivity to heat between B19 and other

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parvoviruses. One explanation is a possible difference in the structure of VP2 because of a partial deletion in the gene for this protein in B19 [13,14]. Further studies are required to understand the mechanism behind this phenomenon, and ensure the safety of blood derivatives.

Generally, measures against viral contamination (e.g. from HIV, HBV, HCV, and B19, etc.) consist of donor screening, viral elimination during processing, nucleic acid amplification tests of final products, and postmarketing surveillance. In our antithrombin preparation, treatments with heat and the Planova 15N filter (15 ± 2 nm; Asahi Kasei Medical Co. Ltd., Tokyo, Japan) were introduced to inactivate/remove the virus, in addition to some other forms of purification which also contribute to viral inactivation/removal [15]. Importantly, a French group reported a case in which B19 was possibly transmitted from an antithrobmin preparation produced by another manufacturer [16]. Although there had been no report of transmission from our antithrombin preparation even before we introduced the use of donor screening and the Planova 15N filter, the data obtained in this study suggest the significance of the stabilizers used for individual products as well as the significance of other steps to eliminate the remaining virus, such as the use of a Planova 15N filter.

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| 販売名<br>（企業名） | ①献血ヴェノグロブリン-IH ヨシトミ（ベネシス）<br>②ヴェノグロブリン-IH（ベネシス）<br>③グロブリン-Wf（ベネシス） | | | | | |

**研究報告の概要**

輸血と関係した新たなvCJD疾患(4例目)が、最近診断された。
この最も新しい患者は、後にvCJDを発症したドナーからの輸血を受けた後、約9年経ってvCJDと診断された。同じドナーからの輸血は、以前に確認された1症例とも関連があった。この患者は未だ生存中であり、専門家の治療を受けている。輸血と関係したこの4番目のvCJD感染症例により、輸血を介したヒトの間におけるvCJD感染リスクが高まっている。4症例は全て、成分輸血に関係したものであり、血漿分画製剤による治療に関連した症例は今まで報告されていない。
患者は、後にvCJDを発生したドナーから、英国で輸血を受けたことが知られている少人数（30人未満）の生存者の内の1人である。これらの全ての人は、すでにvCJDの潜在的な曝露を知らされており、ヘルスケア手法（例えば外科）によってvCJDを他の人々に伝播する機会を減らすための特定の措置をとるよう要請された。Health Protection Agency（HPA）は、後にvCJDを発症したドナーから輸血の曝露を受けた患者のケアを行なっている医師と連絡を取っている。これは、彼らはこの新しい事態を知らされ、最新の情報と輸血によるリスクについての専門家の助言を受けることができるよう保証することを目的としている。
HPA感染症センターの部長であるPeter Borriello教授は、「vCJDのこの新しい症例は、ドネーションの時にはvCJDに感染していたことに気付いていなかったに違いないドナーから輸血を受けた小人数のグループに対するリスクについて、我々の懸念を増大させる。しかし、この新しい症例は、如何なる方法によっても、他の人々のリスクについての我々の理解を変えることはない。それどころか、それは血液によるvCJD感染の伝播リスクを減らすために既にとられている予防措置の重要性を、更に強いものにしている。」と述べた。
NHS血液及び移植部の医学部長であるAngela Robinson博士は、「輸血は、しばしば重篤な患者の、生命を救い又は延命するために行われ、必要時に受ける輸血の利点は、常にリスクに対するバランスが図られなければならない。それにもかかわらず、我々の主な関心は、治療のために使用された血液の品質を維持することを通しての我々の患者の安全性である。」と述べた。
vCJDは、稀な疾患であり、今日まで英国で報告されたvCJD症例の2%未満が、輸血と関係していた。

**使用上の注意記載状況・その他参考事項等**

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 第4例目のvCJDの輸血感染が確認されたとの報告である。<br>これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

**HPA Press Statement**

18 January 2007

## 4th case of variant CJD infection associated with blood transfusion

A new case of variant-Creutzfeldt-Jakob disease (vCJD ) associated with a blood transfusion has recently been diagnosed.

This latest patient has been diagnosed with vCJD about nine years after receiving a blood transfusion from a donor who later went on to develop vCJD. A transfusion from the same blood donor was also associated with one of the previously identified cases. The patient is still alive and is under specialist care.

This fourth case of vCJD infection associated with blood transfusion increases the concern about the risk of vCJD transmission between humans via blood transfusion. All four cases relate to the transfusion of blood components: no cases have been reported relating to treatment with plasma products.

The patient is one of a small number (less than 30) of living individuals who are known to have received a blood transfusion in the UK from a donor who later developed vCJD. All these individuals have previously been informed of their potential exposure to vCJD and asked to take certain precautions to reduce the chance of passing on vCJD to other people via healthcare procedures, such as surgery.

The Health Protection Agency has been in contact with doctors caring for the other patients who have been exposed to blood transfusions from donors who later developed vCJD. This is to ensure that they are informed of this new development and provide access to the latest information and specialist advice about their risk due to blood transfusion.

Professor Peter Borriello, Director of the HPA's Centre for Infections said, "This new case of vCJD infection increases our concern about the risk to the small group of people who had blood transfusions from donors who unknowingly at the time of donation must have had vCJD infection. However, this new case does not change our understanding of the risk for other people in any specific way. It does however reinforce the importance of the precautions that have already been taken to reduce the risk of transmission of vCJD infection by blood."

Dr Angela Robinson, Medical Director of NHS Blood and Transplant said, "Blood transfusions are often given to save or prolong the life of patients who are very ill and the benefit of receiving a transfusion when needed must always be balanced against any possible risk. Nonetheless, our primary concern is the safety of our patients through maintaining the quality of blood used for medical treatment. Since 1997, the NBS has introduced a range of precautionary measures against the risk of vCJD."

vCJD is a rare disease, and less than 2% of the vCJD cases reported to date in the UK have been associated with blood transfusion.

**Notes to Editors:**

1. To date, there have been 66 people identified in the UK who have received vCJD implicated blood transfusions. The transfusions received by these 66 individuals were donated by eighteen different donors who were diagnosed with vCJD after their blood donation. Of these 66 people, 40 have died of illnesses other than vCJD, including one patient who was found to have evidence of vCJD in parts of their body after their death. Including the new (4th) case, 3 of these people who have received vCJD implicated blood transfusions have developed symptoms of vCJD. There are 23 people who have received vCJD implicated blood transfusions who are alive and have not been diagnosed with vCJD.

2. The identification of cases of variant-CJD associated with blood transfusion has depended on the Transfusion Medicine Epidemiology Review, a collaborative study between the National Blood Services, the National CJD Surveillance Unit and the Office of National Statistics. For further information about this study see Hewitt *et al* Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Epidemiology Review study. *Vox Sanguinis* 2006 91:221-230.

3. 'Blood Transfusion' means transfusion with labile blood components (e.g. red cells, platelets, fresh frozen plasma). This latest case (and the previous three referred to) relate to transfusion of blood components and not treatment with plasma products (i.e. products that are manufactured from plasma). To date, no case of vCJD has been associated with treatment with plasma-products (e.g. clotting factors used to treat individuals with bleeding disorders such as haemophilia).

4. This fourth case has been classified by the National CJD Surveillance Unit ( www.cjd.ed.ac.uk ) as a 'probable' case of vCJD. Of the 158 vCJD cases that have died (data to 5 Jan 2007), all 112 that have undergone post-mortem (46 have not) have been 'confirmed' by neuropathological examination (examination of brain tissue).

5. The first clinical case of vCJD associated with transfusion was identified in December 2003. A case of vCJD 'infection' associated with transfusion was identified a few months later. The patient had no symptoms but evidence of infection (abnormal prion proteins) was identified in a post mortem

investigation. The individual died from causes unrelated to vCJD.

6. Following the first case of vCJD associated with a blood transfusion in 2003, the Department of Health asked all recipients of blood transfusions not to donate blood as a precautionary measure to protect the blood supply from vCJD.

7. Patients who are informed that they are considered to be 'at risk' of vCJD for public health purposes are asked to take the following precautions to reduce the chance of passing on vCJD to other people:
   - Not to donate blood, tissues or organs and
   - To inform their healthcare providers of their 'at-risk' status so that special procedures may be arranged for certain instruments used in their healthcare (NB. Their GPs are also asked to do this.)

8. A range of measures have been put in place by the Department of Health to minimise the possible risk of vCJD being passed through blood:
   - Since 1997 all cases of vCJD that are reported to the National CJD Surveillance Unit and diagnosed as having 'probable' vCJD, result in a search of the UK Blood Services blood donor records. If the patient has donated blood, any unused parts of that blood are immediately removed from stock. The fate of all used components of blood from the donor is traced, and surviving recipients informed of their risk.
   - In July 1998, the Department of Health announced that plasma for the manufacture of blood products, such as clotting factors, would be obtained from non-UK sources.
   - Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion.
   - In August 2002 the Department of Health announced that fresh frozen plasma for treating babies and young children born after 1 January 1996 would be obtained from the USA, extended to all children under 16 years of age (Summer 2005).
   - In December 2002, the Department of Health completed its purchase of the largest remaining independent US plasma collector, Life Resources Incorporated. This secures long-term supplies of non-UK blood plasma for the benefit of NHS patients.
   - Since April 2004, blood donations have not been accepted from people who have themselves received a blood transfusion in the UK since 1980. This has been extended to include apheresis donors and donors who are unsure if they had previously had a blood transfusion (August 2004).
   - Since late 2005, blood donations have not been accepted from donors whose blood was transfused to patients who later developed vCJD.
   - The UK Blood Services continue to promote the appropriate use of blood and tissues and alternatives throughout the NHS.

9. The likelihood of a person who may be infected with vCJD going onto develop symptoms of the disease is uncertain, and may depend on individual susceptibility. It is possible that infected individuals may never develop symptoms.

10. For further information contact the HPA press office on 0208 327 7098/7097/6055

11. Specialist care for vCJD is available from The NHS National Prion Clinic, based at The Hospital for Neurology and Neurosurgery, Queen Square, London http://www.nationalprionclinic.org/

12. The National CJD Surveillance Unit is based at the Western General Hospital Edinburgh: www.cjd.ed.ac.uk

13. For further information about vCJD go to:
    http://www.hpa.org.uk/infections/topics_az/cjd/menu.htm
    http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en
    http://www.blood.co.uk/
    http://www.cjd.ed.ac.uk
    http://www.nationalprionclinic.org/

# 医薬品　研究報告 調査報告書

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| 一般的名称 | 輸血 | | 研究報告の公表状況 | http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm | 公表国<br>英国 | |
| 販売名(企業名) | － | | | | | |

**研究報告の概要**

英国において輸血に関連する変異型クロイツフェルト・ヤコブ病（vCJD）の新規症例(4例目)が診断された。
この患者は、後にvCJDを発症したドナーからの輸血を受けて、約9年後にvCJDと診断された。同じドナーからの輸血は、以前に判明した3症例のうちの1例とも関連があった。患者は生存しており、専門医ケアを受けている。
この4例目の輸血関連vCJD感染症例により、輸血を介したヒト-ヒト間でのvCJD伝播のリスクについての懸念が増大している。4症例はすべて血液成分輸血に関連している。血漿製剤投与と関連する症例は報告されていない。
英国健康保護局(HPA)のCentre for InfectionsのDirectorであるPeter Borriello教授は「このvCJD感染症例は、供血時にvCJD感染をしていたドナーからの輸血を受けた、少数の人々へのリスクに関する懸念を増大させる。血液からvCJDが伝播されるリスクを減じるためには、既に講じられている予防措置が重要である。」としている。
vCJDは稀な疾患であり、今までに英国で報告されたvCJD症例の2%未満が輸血と関連づけられている。

エディターの注記
「輸血」とは、血液成分(例えば、赤血球、血小板、新鮮凍結血漿)の輸注を意味する。これまで報告されている4例は血液成分の輸血と関連し、血漿分画製剤(すなわち、血漿から製造される製品)の投与とは関連しない。現在まで、血漿分画製剤(例えば、血友病のような出血性疾患を有する患者を治療するために用いられる凝固因子)の投与に関連づけられたvCJD症例は1例もない。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 英国における輸血による4例目のvCJD症例の報告である。現時点まで血漿分画製剤からのvCJD伝播の報告はない。また、異常プリオン蛋白については、血漿分画製剤の製造工程で除去できるとの考え方がある。 | 今後ともvCJDに関する安全性情報等に留意していく。 |

**使用上の注意記載状況・その他参考事項等**

**重要な基本的注意**
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

**HPA Press Statement**

18 January 2007

## 4th case of variant CJD infection associated with blood transfusion

A new case of variant-Creutzfeldt-Jakob disease (vCJD ) associated with a blood transfusion has recently been diagnosed.

This latest patient has been diagnosed with vCJD about nine years after receiving a blood transfusion from a donor who later went on to develop vCJD. A transfusion from the same blood donor was also associated with one of the previously identified cases. The patient is still alive and is under specialist care.

This fourth case of vCJD infection associated with blood transfusion increases the concern about the risk of vCJD transmission between humans via blood transfusion. All four cases relate to the transfusion of blood components; no cases have been reported relating to treatment with plasma products.

The patient is one of a small number (less than 30) of living individuals who are known to have received a blood transfusion in the UK from a donor who later developed vCJD. All these individuals have previously been informed of their potential exposure to vCJD and asked to take certain precautions to reduce the chance of passing on vCJD to other people via healthcare procedures, such as surgery.

The Health Protection Agency has been in contact with doctors caring for the other patients who have been exposed to blood transfusions from donors who later developed vCJD. This is to ensure that they are informed of this new development and provide access to the latest information and specialist advice about their risk due to blood transfusion.

Professor Peter Borriello, Director of the HPA's Centre for Infections said, "This new case of vCJD infection increases our concern about the risk to the small group of people who had blood transfusions from donors who unknowingly at the time of donation must have had vCJD infection. However, this new case does not change our understanding of the risk for other people in any specific way. It does however reinforce the importance of the precautions that have already been taken to reduce the risk of transmission of vCJD infection by blood."

Dr Angela Robinson, Medical Director of NHS Blood and Transplant said, "Blood transfusions are often given to save or prolong the life of patients who are very ill and the benefit of receiving a transfusion when needed must always be balanced against any possible risk. Nonetheless, our primary concern is the safety of our patients through maintaining the quality of blood used for medical treatment. Since 1997, the NBS has introduced a range of precautionary measures against the risk of vCJD."

vCJD is a rare disease, and less than 2% of the vCJD cases reported to date in the UK have been associated with blood transfusion.

**Notes to Editors:**

1. To date, there have been 66 people identified in the UK who have received vCJD implicated blood transfusions. The transfusions received by these 66 individuals were donated by eighteen different donors who were diagnosed with vCJD after their blood donation. Of these 66 people, 40 have died of illnesses other than vCJD, including one patient who was found to have evidence of vCJD in parts of their body after their death. Including the new (4th) case, 3 of these people who have received vCJD implicated blood transfusions have developed symptoms of vCJD. There are 23 people who have received vCJD implicated blood transfusions who are alive and have not been diagnosed with vCJD.

2. The identification of cases of variant-CJD associated with blood transfusion has depended on the Transfusion Medicine Epidemiology Review, a collaborative study between the National Blood Services, the National CJD Surveillance Unit and the Office of National Statistics. For further information about this study see Hewitt *et al* Creutzfeldt-Jakob disease and blood transfusion: results of the UK Transfusion Epidemiology Review study. *Vox Sanguinis* 2006 91:221-230.

3. 'Blood Transfusion' means transfusion with labile blood components (e.g. red cells, platelets, fresh frozen plasma). This latest case (and the previous three referred to) relate to transfusion of blood components and not treatment with plasma products (i.e. products that are manufactured from plasma). To date, no case of vCJD has been associated with treatment with plasma-products (e.g. clotting factors used to treat individuals with bleeding disorders such as haemophilia).

4. This fourth case has been classified by the National CJD Surveillance Unit ( www.cjd.ed.ac.uk ) as a

'probable' case of vCJD. Of the 158 vCJD cases that have died (data to 5 Jan 2007), all 112 that have undergone post-mortem (46 have not) have been 'confirmed' by neuropathological examination (examination of brain tissue).

5. The first clinical case of vCJD associated with transfusion was identified in December 2003. A case of vCJD 'infection' associated with transfusion was identified a few months later. The patient had no symptoms but evidence of infection (abnormal prion proteins) was identified in a post mortem investigation. The individual died from causes unrelated to vCJD.

6. Following the first case of vCJD associated with a blood transfusion in 2003, the Department of Health asked all recipients of blood transfusions not to donate blood as a precautionary measure to protect the blood supply from vCJD.

7. Patients who are informed that they are considered to be 'at risk' of vCJD for public health purposes are asked to take the following precautions to reduce the chance of passing on vCJD to other people:
   - Not to donate blood, tissues or organs and
   - To inform their healthcare providers of their 'at-risk' status so that special procedures may be arranged for certain instruments used in their healthcare (NB. Their GPs are also asked to do this.)

8. A range of measures have been put in place by the Department of Health to minimise the possible risk of vCJD being passed through blood:
   - Since 1997 all cases of vCJD that are reported to the National CJD Surveillance Unit and diagnosed as having 'probable' vCJD, result in a search of the UK Blood Services blood donor records. If the patient has donated blood, any unused parts of that blood are immediately removed from stock. The fate of all used components of blood from the donor is traced, and surviving recipients informed of their risk.
   - In July 1998, the Department of Health announced that plasma for the manufacture of blood products, such as clotting factors, would be obtained from non-UK sources.
   - Since October 1999, white blood cells (which may carry the greatest risk of transmitting vCJD) have been removed from all blood used for transfusion.
   - In August 2002 the Department of Health announced that fresh frozen plasma for treating babies and young children born after 1 January 1996 would be obtained from the USA, extended to all children under 16 years of age (Summer 2005).
   - In December 2002, the Department of Health completed its purchase of the largest remaining independent US plasma collector, Life Resources Incorporated. This secures long-term supplies of non-UK blood plasma for the benefit of NHS patients.
   - Since April 2004, blood donations have not been accepted from people who have themselves received a blood transfusion in the UK since 1980. This has been extended to include apheresis donors and donors who are unsure if they had previously had a blood transfusion (August 2004).
   - Since late 2005, blood donations have not been accepted from donors whose blood was transfused to patients who later developed vCJD.
   - The UK Blood Services continue to promote the appropriate use of blood and tissues and alternatives throughout the NHS.

9. The likelihood of a person who may be infected with vCJD going onto develop symptoms of the disease is uncertain, and may depend on individual susceptibility. It is possible that infected individuals may never develop symptoms.

10. For further information contact the HPA press office on 0208 327 7098/7097/6055

11. Specialist care for vCJD is available from The NHS National Prion Clinic, based at The Hospital for Neurology and Neurosurgery, Queen Square, London http://www.nationalprionclinic.org/

12. The National CJD Surveillance Unit is based at the Western General Hospital Edinburgh: www.cjd.ed.ac.uk

13. For further information about vCJD go to:
    http://www.hpa.org.uk/infections/topics_az/cjd/menu.htm
    http://www.dh.gov.uk/PolicyAndGuidance/HealthAndSocialCareTopics/CJD/fs/en
    http://www.blood.co.uk/
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    http://www.nationalprionclinic.org/

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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**研究報告の概要**

○赤血球中の病原体のS-303およびグルタチオンを用いた改良処理法による不活化

背景:S-303 (アクリジン化合物) 0.2mMおよびグルタチオン(GSH) 2.0mMを用いた赤血球(RBC)の処理により、ウイルス、細菌、寄生虫を含む様々な病原体および混入白血球を不活化することが示されている。PhaseIII臨床試験中、S-303処理RBCの表面に結合した残存アクリジンに特異的な抗体が数名の被験者に検出され、免疫原性を低減するために改良S-303処理法が開発された。改良された工程ではS-303 0.2mMおよびGSH 20mMを用いる。ここでは改良S-303処理法を用い、RBC中の細菌およびウイルス不活化の有効性を評価するためにデザインした現行試験から得られたデータを報告する。

方法:AS-3保存液中に白血球除去したRBC約280 mL(ヘマトクリット約60%)を調製した。RBCに約6logs/mLの病原体を添加し、処理を施していない病原体添加コントロールを分取した。これに、GSHを加えてよく攪拌した。室温で5～10分間インキュベートした後、サンプルを取り出し、残存生菌を検出する分析を行った。コントロール溶液は、調製直後に力価を測定し、その後病原体添加3時間後に再度力価測定した。試験には輸血に関連するグラム陽性菌およびグラム陰性菌、マイナス鎖RNAウイルスとしてVesicular stomatisウイルス、非エンベロープDNAウイルスとしてAdenovirus 5を用いた。また、HIVおよびウシウイルス性下痢ウイルス(HCVのモデル)の不活化を評価した。各微生物について反復実験を実施し、得られた結果を観察された不活化レベルの平均±標準偏差(SD)で示した。不活化はlog reductionで表した。

結果:グラム陽性菌およびグラム陰性菌、エンベロープ、非エンベロープウイルスのいずれも改良S-303処理により十分に不活化された。

結論:当該改良S-303処理は、RBC中の細菌およびウイルス病原体を効果的に不活化する。

| 報告企業の意見 | 今後の対応 |
|---|---|
| S-303およびグルタチオンを用いた改良処理法によって赤血球製剤中の細菌およびウイルス病原体が効果的に不活化されたとの報告である。 | 日本赤十字社では、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起している。また、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知する。<br>今後も細菌やウイルスを不活化する方策について情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

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vCJD等の伝播のリスク

*Blood* (ASH Annual Meeting Abstracts) 2006 108: Abstract 4144
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# Inactivation of Pathogens in Red Blood Cells by a Modified Treatment Process Utilizing S-303 and Glutathione.

**L. Sawyer[1,*], R. Mababangloob[1,*], N. Patel[1,*], J. Kinsey[1,*], A. Sampson-Johannes[1,*] and D. Hanson[1,*]**

(Intr. by Laurence Corash) [1] Development, Cerus Corporation, Concord, CA, USA.

## Abstract

Background: Treatment of red blood cells (RBC) with 0.2 mM S-303 (an acridine compound) and 2.0 mM glutathione (GSH) has previously been shown to inactivate a variety of pathogens, including viruses, bacteria, protozoan parasites and contaminating leukocytes. During Phase III clinical trials antibodies to S-303-treated RBC were detected in a few trial participants. These antibodies were later determined to be specific to residual acridine bound to the RBC surface. As a result of these antibodies, a modification of the S-303 treatment process has been developed to reduce the potential immunogenicity of S-303-treated RBC. This modified process utilizes 0.2 mM S-303 and 20 mM GSH. In this abstract we report data from an on-going series of studies designed to evaluate the efficacy of bacterial and viral inactivation in RBC using the modified S-303 treatment process.

Methods: Leukoreduced RBC units of approximately 280 mL with a hematocrit of approximately 60% were prepared in AS-3 storage medium. RBC units were inoculated with approximately 6 logs/mL of organism when possible, and an aliquot was removed to serve as the untreated, input control. GSH in a solution of 2 equivalents NaOH was added to the inoculated units to a final concentration of 20 mM and mixed well. Following a 5 to 10-minute room temperature incubation, S-303 was added to a final concentration of 0.2 mM and the units were again mixed well and incubated at 20 to 25 °C for three hours. After incubation, samples were removed and assayed to detect residual viable organisms. Control preparations were titered immediately after preparation and again after the 3-hour incubation. Bacterial titers were determined by colony formation on agar plates and viral titers were determined by plaque formation in MT-2 cells (HIV), BT cells (BVDV), vero E6 cells (VSV) and A549 cells (Adenovirus). Bacteria were chosen to represent Gram positive and Gram negative organisms of relevance to transfusion. Vesicular stomatis virus (VSV) was used as a representative negative sense RNA virus, and Adenovirus 5 as a representative non-enveloped DNA virus. HIV and bovine viral diarrhea virus (BVDV, model for HCV) inactivation was evaluated because of their relevance to blood transfusion. Replicate experiments were performed for each organism and the results are expressed as the mean and standard deviation (SD) of the observed inactivation levels. Inactivation is expressed as log reduction.

Results: See table below. Both Gram positive and Gram negative bacteria were effectively inactivated by treatment with the modified S-303 process. This process also resulted in effective inactivation of both the enveloped and non-enveloped viruses tested.

Conclusion: The modified S-303 process effectively inactivates bacterial and viral pathogens in RBC.

| Organism | Log Reduction[a] (Mean ±SD) |
|---|---|
| Staphylococcus aureus | ≥6.9 ±0.4 |
| Staphylococcus epidermidis | ≥7.0 ±0.1 |
| Yersinia enterocolitica | 4.1 ±0.8 |
| Escherichia coli | 6.1 ±0.7 |
| Serratia marcescens | 4.0 ±0.4 |
| HIV | >5.8 ±0.2 |
| BVDV | >4.4 ±0.2 |
| Vesicular stomatitis virus (VSV) | 4.2 ±0.1 |
| Adenovirus 5 | ≥8.0 ±0.3 |

## Footnotes

[a] Corresponding author

**Disclosures:** Cerus Corporation Employee.; Cerus Corporation Stock Options.

# 医薬品 研究報告 調査報告書

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**研究報告の概要**

○フィリピンでイヌにかまれた京都の男性が狂犬病で死亡
2006年11月17日、京都府の保健所は、京都市の60歳代の男性がフィリピンで犬にかまれ、帰国後に狂犬病を発症して死亡したと発表した。
厚労省によると、日本人が国内で狂犬病を発症したのは36年ぶりである。
厚労省によると、男性はフィリピン滞在中の8月末に野良犬にかまれ、11月1日に帰国した。9日に風邪のような症状で京都市内の病院を受診した。その後、幻覚症状、水や風を怖がるなど狂犬病特有の症状を発症した。国立感染症研究所が調べたところ、男性の唾液から狂犬病ウイルスが検出された。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 京都市の60歳代の男性がフィリピンで犬にかまれ、帰国後に狂犬病を発症して死亡したとの報告である。 | 日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、動物に噛まれた場合は3ヶ月間供血不可としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Back

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**Subject** PRO/AH/EDR> Rabies, human - Japan (Kyoto) ex Philippines

RABIES, HUMAN - JAPAN (KYOTO) EX PHILIPPINES
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Date: Sat 18 Nov 2006
From: Akira Goto <dolphin@mail.ne.jp>
Source: Daily Yomiuri online, Sat 18 Nov 2006 [edited]
<http://www.yomiuri.co.jp/dy/national/20061118TDY02001.htm>

A Kyoto resident in his 60's died of rabies on his return to Japan after a stray dog bit his hand in the Philippines, the Public Health and Welfare Bureau in Kyoto announced on Fri 17 Nov 2006. The man, who fell into a coma before his death, was the first Japanese to be diagnosed with the virus in 36 years, the Health, Labor and Welfare Ministry said.

According to the ministry, the man was bitten by the dog at the end of August [2006] and returned to Japan on 1 Nov 2006. He visited a doctor in Kyoto on 9 Nov 2006 with cold [flu-like?] symptoms before developing characteristic signs of rabies such as hallucinations and a fear of water and wind.

The National Institute of Infectious Diseases diagnosed the man with rabies after testing his saliva.

--
Akira Goto
<dolphin@mail.ne.jp>

[Akira Goto has provided the following additional information translated from a press release in Japanese from the Ministry of Health, Labour and Welfare (<http://www.mhlw.go.jp/houdou/2006/11/h1116-2.html>). "The 1970 human case was also an imported case from Nepal; the most recent domestic human and canine rabies cases in Japan were reported in 1954 and 1956, respectively."

ProMED-mail greatly appreciates Akira Goto's initiative in providing this information. - Mod.CP]

[see also:
2002
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Rabies, imported dogs - Japan: alert 20021229.6150]
.....cp/pg/dk

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# Kyoto man bitten by dog in R.P. dies of rabies

The Yomiuri Shimbun

A Kyoto man in his 60s died of rabies on his return to Japan after a stray dog bit his hand in the Philippines, the Public Health and Welfare Bureau in Kyoto said Friday.

The man, who fell into a coma before his death, was the first Japanese to be diagnosed with the virus in 36 years, the Health, Labor and Welfare Ministry said.

According to the ministry, the man was bitten by the dog at the end of August and returned to Japan on Nov. 1. He visited a doctor in Kyoto on Nov. 9 with cold symptoms before developing characteristic signs of rabies such as hallucinations and a fear of water and wind.

The National Institute of Infectious Diseases diagnosed the man with rabies after testing his saliva.

(Nov. 18, 2006)

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医薬部外品　　研究報告　調査報告書
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| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2006 年 12 月 6 日 | 新医薬品等の区分 | 厚生労働省処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 研究報告の公表状況 | NIKKEI NET/20061205 | 公表国<br>日本 | | |
| 販売名<br>(企業名) | ①献血アルブミン-Wf（ベネシス）<br>②献血アルブミン(5%)-Wf（ベネシス）<br>③アルブミン-Wf（ベネシス）<br>④コンコエイトーHT（ベネシス）<br>⑤クリスマシンーM（ベネシス） | | | | | |

**研究報告の概要**

既存の治療薬がほとんど効かず、世界保健機関（WHO）が警戒を呼び掛けている「超多剤耐性」の結核菌が、国内でも入院患者の 0.5%から検出されたことが、結核研究所の調査で 5 日までに分かった。
結核菌は、薬の服用を途中でやめるなど誤った治療で耐性が生じやすく、既に国内でも問題になっているが、それより治療が格段に難しい超耐性菌の確認は初めて。
検出例の半数は薬の服用歴がなかったことから、他の患者から感染した可能性が高い。調査した同研究所の御手洗聡細菌検査科長は「菌の耐性の強さに応じた個室療養を可能にするような治療態勢の見直しが必要だ」と指摘している。
御手洗科長らは、2002 年 6―11 月にかけて国内 99 の結核治療施設の入院患者 3122 人から採取した結核菌を分析した。
効き目が強い 2 種類の第 1 選択薬がともに効かない多剤耐性菌が 55 人(全体の 1.8%)に見つかったが、うち 17 人(同 0.5%)から検出された菌は、補助的に用いられる第 2 選択薬も複数の種類が効かない超多剤耐性菌だった。

**使用上の注意記載状況・その他参考事項等**

代表として献血アルブミン-Wf の記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している.更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人アルブミンを精製し、アルブミン濃度 25w/v%に調整した製剤であり、ウイルス不活化を目的として、製造工程において 60℃、10 時間の液状加熱処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 既存の治療薬がほとんど効かず、世界保健機関（WHO）が警戒を呼び掛けている「超多剤耐性」の結核菌が、国内からも検出されたとの報告である。<br>血漿分画製剤からの結核菌伝播の事例は報告はされていない。万一原料血漿に結核菌が混入したとしても、除菌ろ過等の製造工程にて除去されるものと考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

BENE2006-026



# 「超耐性」結核菌を国内で確認、入院患者の0.5%で検出

既存の治療薬がほとんど効かず、世界保健機関(WHO)が警戒を呼び掛けている「超多剤耐性」の結核菌が、国内でも入院患者の0.5%から検出されたことが、結核研究所の調査で5日までに分かった。

結核菌は、薬の服用を途中でやめるなど誤った治療で耐性が生じやすく、既に国内でも問題になっているが、それより治療が格段に難しい超耐性菌の確認は初めて。

検出例の半数は薬の服用歴がなかったことから、他の患者から感染した可能性が高い。調査した同研究所の御手洗聡細菌検査科長は「菌の耐性の強さに応じた個室療養を可能にするような治療態勢の見直しが必要だ」と指摘している。

御手洗科長らは、2002年6—11月にかけて国内99の結核治療施設の入院患者3122人から採取した結核菌を分析した。

効き目が強い2種類の第1選択薬がともに効かない多剤耐性菌が55人(全体の1.8%)に見つかったが、うち17人(同0.5%)から検出された菌は、補助的に用いられる第2選択薬も複数の種類が効かない超多剤耐性菌だった。

[2006年12月5日/共同]

別紙様式第2

別紙(3)

# 医薬品　研究報告　調査報告書

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|---|---|---|---|---|---|---|
| 識別番号・報告回数 | | | 報告日 | 第一報入手日<br>2006年10月16日 | 新医薬品等の区分<br>該当なし | 厚生労働省処理欄 |
| 一般的名称 | | | 研究報告の公表状況 | Continuing Mycobacterium bovis transmission from animals to humans in New Zealand Epidemiology and Infection 2006,134/5 (1068-1073) | 公表国<br>英国 | |
| 販売名（企業名） | タココンブ（CSL ベーリング株式会社） | | | | | |

**研究報告の概要**

問題点（ニュージーランドにおける動物から人への Mycobacterium bovis 伝播）

M. bovis はニュージーランドの野生動物や家畜の牛などで一般的な感染症である。M. bovis は結核菌による疾患と臨床上区別できない疾病を起こす。今回の研究で、現在のニュージーランドのヒトにおける M. bovis 感染の疫学を述べ、1995 年から 2002 年の間の疫学調査や臨床検査結果から M. bovis 感染の可能な感染源を調査した。

当該調査期間中に 54 例の M. bovis 感染が確認され、年平均 6.8 例であった。その中の 34 例を詳細に検討できた。年平均 M. bovis 感染率を性別、年齢別に比較すると、60-69 歳の群が最も高率で、次に 70 歳以上の群であった。男女比は、3:1 であった。出生国はニュージーランドが 71.9%で、残りの 28.9%はニュージーランドに移ってきていた。感染部位は呼吸器感染症が 56.7%あった。その他に腹膜、リンパ節、腕・手首などであった。73.1%の感染者は入院した。ヒトから分離された M. bovis の REA タイプの 61%が、牛などから分離されたタイプと同一であった。

M. bovis 感染は 1940 年代のミルク加熱処理導入前の汚染ミルクにより頻繁に生じ、大部分が肺以外の感染であった。

ニュージーランドにおいて、動物からヒトに低レベルで持続的な M. bovis 感染があることを示していることが判った。

本研究でヒトが M. bovis 感染源であることを除外することができなかったが、ヒトは一般的に重要な感染源ではない。

使用上の注意記載状況・その他参考事項等

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本成分のトロンビンは、牛血液を原料としている。牛がM. bovisに感染していたとしても、本剤の製造工程で不活化されると考えられる。 | 今後とも新しい感染症に関する情報収集に努める所存である。 |



62

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# Continuing *Mycobacterium bovis* transmission from animals to humans in New Zealand

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## SUMMARY

New Zealand has a large reservoir of *Mycobacterium bovis* infection in wild and farmed animals. This study aimed to assess the extent of human infection with this organism and the potential contribution of these animal sources. Combined epidemiological and laboratory investigation of human tuberculosis cases over the period 1995–2002 showed that *M. bovis* accounted for 2·7% (54/1997) of laboratory-confirmed human tuberculosis cases, a rate of 0·2/100 000 population. *M. bovis* isolates from humans (23) were typed using restriction endonuclease analysis (REA) and compared with isolates from wild and domestic animals (2600). Fourteen (61%) of the human isolates had REA patterns that were identical to patterns for isolates from cattle, deer, possums, ferrets, pigs, and occasionally cats. These results suggest a low level of ongoing *M. bovis* transmission from animal reservoirs to humans in New Zealand.

## INTRODUCTION

*Mycobacterium bovis* can cause disease in humans that clinically is indistinguishable from that due to *Mycobacterium tuberculosis*. Although now uncommon in most developed countries, *M. bovis* remains an important cause of tuberculosis in developing countries [1, 2]. *M. bovis* is a common infection in wild animals in New Zealand, notably introduced Australian brushtail possums (*Trichosurus vulpecula*), and these occasionally infect farmed cattle and deer [3]. This situation raises the potential for animal-to-human transmission of this pathogen.

A previous review of human infection with *M. bovis* in New Zealand, identified 60 cases over the period from January 1985 to July 1995 [4]. The majority of these cases occurred in elderly people and probably represent infections acquired before introduction of milk pasteurization and herd testing in the 1940s and 1950s. However, this total included some younger cases (15 under 40 years of age) who should not have been exposed to unpasteurized milk.

This study, therefore, aimed to describe the current epidemiology of human *M. bovis* infection in New Zealand and determine the possible sources of these infections using combined epidemiological and laboratory investigation of cases over the 1995–2002 period.

## METHODS

Human *M. bovis* and *M. tuberculosis* infections from 1995 to 2002 were identified using notification records supplemented by laboratory detection data from New Zealand's three tuberculosis referral laboratories. Cases occurring from 1998 to 2002 were analysed in

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detail, including key demographic and risk factor information. This period was chosen because few human isolates were stored before 1998. Rates were calculated using population data from the 2001 Census. Epi-Info (CDC, Atlanta, GA, USA) was used to calculate crude rate ratios (RR) and 95% confidence intervals 95% CI and the $\chi^2$ test for linear trend.

The three tuberculosis laboratories sent all stored human *M. bovis* isolates to AgResearch, Wallaceville for DNA fingerprinting. AgResearch carried out this characterization of human isolates using restriction endonuclease analysis (REA). Each isolate was separately digested with three restriction enzymes, *Bst*EII, *Pvu*II and *Bcl*I. This process generated three gels and associated band pattern for each isolate. This was the same method as had been used to type 2600 *M. bovis* isolates collected from a wide variety of domestic and wild animals in New Zealand over the 1982–2003 period [5–7]. These animal isolates had been characterized into 270 different REA types and the information stored in a DNA database. The restriction types of the human *M. bovis* isolates were compared to these animal types.

The epidemiological information was analysed in conjunction with the results of the DNA fingerprinting of the *M. bovis* strains recovered from the patients. This process aimed to determine the likelihood of the human infections being acquired from specific animal exposures in New Zealand or from overseas.

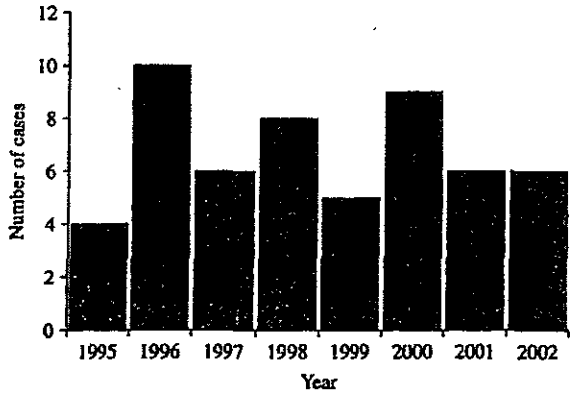


**Fig. 1.** Human *M. bovis* infections in New Zealand by year, 1995–2002.




**Fig. 2.** Human *M. bovis* infection incidence by age and sex, average annual rate per 100 000 population, 1998–2002.

## RESULTS

### Epidemiology of *M. bovis* infection

Notification and laboratory data identified a total of 54 human cases of *M. bovis* (excluding *M. bovis* BCG) infection from 1995 to 2002. There were a further 1943 cases of infection with *M. tuberculosis*, so *M. bovis* represented 2·7% (54/1997) of the laboratory-confirmed tuberculosis cases detected over that period.

The incidence of newly diagnosed *M. bovis* infections (Fig. 1) did not increase over the period 1995–2002 ($\chi^2$ for linear trend, $P=1{\cdot}0$). The average number of bovine tuberculosis cases in the 8-year period was 6·8 per year.

The 34 *M. bovis* infections occurring from 1998 to 2002 were reviewed in detail. Over this 5-year period, cases occurred in 16 of New Zealand's 21 district health board areas, with an average rate of 0·18/100 000 cases. Rates ranged from 0 to 1·32/100 000, although these figures are based on small numbers. Areas reporting rates above the national average were West Coast, Wairarapa, Hutt, Lakes, Tairawhiti, Waikato, Counties Manukau, Eastern Bay of Plenty, Capital Coast and Whanganui districts.

Figure 2 shows the average annual rates of *M. bovis* infection by age group and sex. Highest rates were in the 60–69 years age group, followed by those ⩾70 years of age. Gender was recorded for all 34 cases. Male cases outnumbered females by 3:1.

Country of birth information was available for 32 cases of whom 23 (71·9%) were born in New Zealand and the remaining nine (28·1%) had migrated to New Zealand. Six of the immigrant cases had been born in developing countries. The rates for Maori (2·1/100 000) and Pacific people (1·5/100 000) and people of other ethnicity (1·2/100 000) were higher than for Europeans (0·5/100 000).

The site of infection was recorded for 88·2% of cases (30/34). Pulmonary infection was the most common site, accounting for 56·7% (17/30). The remaining extra-pulmonary sites included: peritoneum,

Table 1. *Comparison of the epidemiological characteristics of human* M. bovis *and* M. tuberculosis *infections, 1998–2002*

| Characteristic | *M. bovis* infection (% recorded) | *M. tuberculosis* infection (% recorded) |
|---|---|---|
| Sex | | |
| Male | 25 (73·5) | 696 (52·4) |
| Female | 9 (26·5) | 632 (47·6) |
| Median age (yr) | 57 | 37 |
| Age group (yr) | | |
| 0–19 | 1 (2·9) | 153 (11·5) |
| 20–59 | 17 (50·0) | 866 (64·9) |
| ⩾60 | 16 (47·1) | 316 (23·7) |
| Ethnicity | | |
| European | 14 (45·2) | 169 (13·1) |
| Maori | 11 (35·5) | 217 (16·8) |
| Pacific | 3 (9·7) | 240 (18·6) |
| Other | 3 (9·7) | 665 (51·5) |
| Region | | |
| North Island | 26 (76·5) | 1183 (88·5) |
| South Island | 8 (23·5) | 153 (11·5) |
| Site | | |
| Pulmonary | 17 (56·7) | 685 (56·9) |
| Non-pulmonary | 10 (33·3) | 383 (31·8) |
| Both pulmonary and non-pulmonary | 3 (10·0) | 134 (11·1) |
| Birth | | |
| Inside New Zealand | 23 (71·9) | 360 (29·6) |
| Outside New Zealand | 9 (28·1) | 855 (70·4) |
| Total 1998–2002 | 34 | 1336 |

lymph nodes, arm/wrist (tenosynovitis), genitourinary system, hip joint, and proximal interphalangeal joint. Most cases were hospitalized (19/26 or 73·1%). The case- fatality rate was 15·4% (4/26).

### Comparison of *M. bovis* and *M. tuberculosis* infection

Table 1 compares the characteristics of *M. bovis* and *M. tuberculosis* cases for the 5-year period 1998–2002. People infected with *M. bovis* were significantly more likely to be male (RR 2·5, 95% CI 1·2–5·3), over 60 years of age (RR 2·5, 95% CI 1·2–5·3), European (RR 6·2, 95% CI 3·1–12·1) or Maori (RR 2·2, 95% CI 1·1–4·6), to have been born inside New Zealand (RR 4·6, 95% CI 2·0–10·6) rather than migrated there, and to be living in the South Island at the time of diagnosis (RR 2·3, 95% CI 1·1–5·0). Of note is that *M. bovis* infection was not more commonly associated with extra-pulmonary sites of infection than was *M. tuberculosis*.

### Laboratory investigation of human *M. bovis* infections

The three tuberculosis referral laboratories located 23 viable human *M. bovis* isolates for the 1998 to 2002 period, out of a possible total of 34. The AgResearch laboratory at Wallaceville typed these isolates and compared their patterns to REA results for animal isolates held in their database (Table 2).

Eight isolates were classified as 'foreign' strains as their REA pattern has not been seen previously in a New Zealand animal isolate. Of this group, four of the source patients were recorded as having been born overseas (one in Fiji, one in the Cook Islands, one in England and one in Scotland), and four were born in New Zealand. One isolate was the strain (AN5) used for making bovine purified protein derivative (PPD). This infection was a result of an accidental exposure that occurred in a laboratory manufacturing tuberculin.

This left 14 isolates that had an REA pattern that was identical to one that has been seen previously in wild or domestic animals in New Zealand. These isolates included nine different unique REA patterns. Of these, five isolates had the same REA type (115) that is found very commonly in the Central North Island in a wide range of animal reservoirs. Two isolates had the same type (21) that is found very commonly in the Wairarapa and on the West Coast of the South Island, again in a wide range of reservoir animals. The remaining seven REA types were all represented by a single isolate. The types found in humans had all been isolated from bovine reservoirs (14/14 isolates). There was also a high degree of overlap with those found in deer (12/14), ferrets (10/14) and possums (11/14).

Of these 14 cases with REA patterns that matched specimens from animals in New Zealand, eight had risk factor information recorded. Of these, five reported animal contact that could potentially have given rise to infection. These contacts included living or working on a farm, being an abattoir worker, consumption of unpasteurized milk, and working as a veterinary receptionist. One case was infected following accidental inoculation during necropsy of a tuberculous possum, as specifically reported elsewhere [8].

## DISCUSSION

Findings from this study suggest a continuing low level of *M. bovis* transmission from animals to humans in New Zealand. Although *M. bovis* is a relatively uncommon cause of human tuberculosis in New

Table 2. *Human* M. bovis *isolates (1998–2002), and animal isolates (1982–2003) by REA type (see references [5–7] for details of REA types)*

| REA type | No. of human isolates (% of total human isolates) | No. of animal isolates | No. of isolates from each animal species (% of total isolates for that species) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Bovine | Cervine | Ferret | Possum | Porcine | Feline |
| Foreign* | 8 (34·8) | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 115 | 5 (21·7) | 417 | 308 (24·1) | 51 (10·6) | 15 (7·7) | 27 (6·4) | 15 (8·1) | 1 (2·4) |
| 21 | 2 (8·7) | 97 | 41 (3·2) | 15 (3·1) | 11 (5·6) | 18 (4·3) | 11 (5·9) | 1 (2·4) |
| AN5* | 1 (4·3) | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| 6 | 1 (4·3) | 3 | 3 (0·2) | 0 | 0 | 0 | 0 | 0 |
| 9 | 1 (4·3) | 3 | 2 (0·2) | 1 (0·2) | 0 | 0 | 0 | 0 |
| 19 | 1 (4·3) | 33 | 7 (0·5) | 24 (5·0) | 1 (0·5) | 1 (0·2) | 0 | 0 |
| 27 | 1 (4·3) | 30 | 1 (0·1) | 3 (0·6) | 0 | 13 (3·1) | 13 (7·0) | 0 |
| 62 | 1 (4·3) | 109 | 50 (3·9) | 11 (2·3) | 16 (8·2) | 10 (2·4) | 17 (9·2) | 5 (11·9) |
| 151 | 1 (4·3) | 111 | 78 (6·1) | 18 (3·7) | 7 (3·6) | 6 (1·4) | 1 (0·5) | 1 (2·4) |
| 158 | 1 (4·3) | 3 | 3 (0·2) | 0 | 0 | 0 | 0 | 0 |
| Other | 0 | 1794 | 783 (61·4) | 359 (74·5) | 146 (74·5) | 344·(82·1) | 128 (69·2) | 34 (81·0) |
| Total | 23 | 2600 | 1276 | 482 | 196 | 419 | 185 | 42 |

REA, Restriction endonuclease analysis; n.a., not applicable.
* Never found in animals in New Zealand.

Zealand, accounting for about 2·7% of human disease, this contribution is higher than that seen in other developed countries. In Australia *M. bovis* caused ~1% of human disease over the 1970–1994 period [9] and in Britain it accounted for 1·2% of cases from 1986 to 1990 [10]. Another indication of *M. bovis* transmission within New Zealand comes from the observation that most cases (71·9% over the 1998–2002 period) were born in New Zealand. This proportion is significantly higher than that seen for infection with *M. tuberculosis* where a minority of cases are New Zealand born (only 29·6% during this period).

There has been little increase over 17 years in the incidence of human infection with *M. bovis* in New Zealand. A previous analysis by Richard Pooley identified 60 cases over the $11\frac{1}{2}$-year period from January 1985 to July 1995, an average of 5·2 cases a year [4]. This present study identified an average of 6·8 cases a year for the 8-year period from 1995 to 2002.

The site of tuberculosis infection gives an indication of the possible route of transmission. The majority (56·7%) of *M. bovis* infections are pulmonary, which is a similar proportion to that seen for *M. tuberculosis* infection. This contrasts with the pattern prior to pasteurization of milk in the 1940s when infection was frequently transmitted via contaminated milk and the majority of infections were non-pulmonary [11].

The ages of human *M. bovis* cases suggest that many are reactivations of old infections rather than newly acquired disease. Among the New Zealand-born cases, most (80%) were >30 years of age which is similar to the 85% found by Pooley [4]. The median age of *M. bovis* infections (57 years) is much older than that for *M. tuberculosis* infection (37 years). Despite this, there are still *M. bovis* infections occurring in relatively young New Zealanders with four <30 years of age at the time of diagnosis. They were all born after control measures were introduced to limit transmission through contaminated milk. These measures included the compulsory testing of dairy cattle from 1961 and steady introduction of milk pasteurization from the 1940s to the early 1970s. These cases are likely to have acquired infection from other sources. The 3:1 preponderance of males compared to females suggests that occupational settings may be important sources of *M. bovis* exposure. A similar overrepresentation of males is seen for leptospirosis in New Zealand, and has been attributed to the larger proportion of males working as farm and abbatoir workers where most exposure to this pathogen occurs [12].

Combined results of molecular and epidemiological investigation can provide evidence of the sources of human infections. For the two *M. bovis* infections resulting from well-documented occupational exposures, the source of infection was clearly identified. Five other cases in this present study reported animal contact that could potentially have given rise to

infection and had REA patterns that were the same as ones found in wild and domestic animals in New Zealand. Here the evidence is suggestive of local infection but more detailed investigation would be required to identify specific sources. Far less can be concluded about the source of infection for the remaining cases, including the eight others with REA patterns that were the same as ones found in wild and domestic animals in New Zealand.

There are few reported studies that have used molecular methods to investigate sources of human *M. bovis* infection. One study analysed 20 human and 58 animal isolates from Argentina and 47 human and 13 animal isolates from The Netherlands using restriction fragment length polymorphism (RFLP) patterns probed by the insertion element IS*6110* [13]. It found that most human isolates in Argentina were of a single type that also predominated among cattle isolates. This situation contrasted with The Netherlands where the *M. bovis* fingerprints of strains from humans were very diverse and none were found among *M. bovis* strains of animal origin. A study in Sweden used similar methods to characterize isolates from 11 humans and 28 farmed deer sampled from an outbreak affecting five herds. The *M. bovis* fingerprints of the human isolates were diverse, whereas the isolates from the deer all had a different and very specific pattern [14]. The molecular epidemiology of human *M. bovis* infection in New Zealand, characterized by human infection with a diverse range of REA types, resembles the situation seen in The Netherlands and Sweden more than that in Argentina, but differs from Sweden and Argentina in having many of these types infect both animals and humans.

This study cannot exclude human sources for some *M. bovis* infections. For example, one case in our series reported contact with another case of human *M. bovis* infection. Human-to-human [15], and even human-to-animal transmission of *M. bovis* has been documented [16], and a recently reported contact investigation study suggests that in some circumstances *M. bovis* may be almost as efficient at human-to-human transmission as *M. tuberculosis* [17]. Despite these observations, humans are usually a 'dead-end' host for *M. bovis* infections and human contact is not generally an important source of infection.

The major limitations of this present study are its small size and the lack of complete epidemiological information on human cases. Continuing the typing of human isolates prospectively would increase the size of this study sample over time. It would also be useful to undertake more detailed epidemiological investigation of these human cases, including their history of animal contact and geographic areas where they have lived in the past, so that these results could be compared with the extensive information held on *M. bovis* distribution in animals. A case-control study should also be considered to provide a more precise estimate of the risk of human infection associated with specific forms of animal contact.

## ACKNOWLEDGEMENTS

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## DECLARATION OF INTEREST

None.

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# 医薬品
# 医薬部外品　研究報告　調査報告書
# 化粧品

| 識別番号・報告回数 | | 回 | 報告日<br>年　月　日 | 第一報入手日<br>2006 年 10 月 3 日 | 新医薬品等の区分<br>該当なし | 総合機構処理欄 |
|---|---|---|---|---|---|---|
| 一般的名称 | | | 研究報告の公表状況 | Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products<br>Miriam E.R. et. al<br>Transfusion. 46; 1770-77 | 公表国<br>米国 | |
| 販売名（企業名） | | | | | | |

**研究報告の概要**

背景:
重症急性呼吸器症候群コロナウイルス (SARS-CoV) が感染患者の血液内で検出された。このことから，献血や血漿分画製剤の汚染の可能性が考えられる。この報告書ではタンパク溶液中の SARS-CoV 不活化に有効な方法を報告している。
研究方法:
熱，紫外線 (UV) 照射法，オクタン酸，及び有機溶媒/界面活性 (S/D) 処理が，擬似的な血漿分画製剤タンパク溶液中の SARS-CoV に対し不活化効果を示すか実験を行った。処理サンプルにおける組織培養増殖アッセイを行い，不活化効果を評価した。
結果:
60 度の熱処理では SARS-CoV の不活化に 15-30 分必要であった。UVC (200-280nm) は 40 分で効果的に SARS-CoV を不活化したが，一方，UVA (320-400nm) ではウイルス不活化を高めるためにソラーレンの添加が必要であった。ウシ血清アルブミンは UVC と UVA の SARS-CoV の不活化能力を制限し，オクタン酸処理はスパイクされた溶液中の SARS-CoV 感染力を低減しなかった。S/D 処理では，Triton X-100, Tween80，及びナトリウムコール酸で SARS-CoV を不活化するのにそれぞれ 2，4，及び 24 時間必要であった。
結論:
事実上，熱，UVC 照射法，及び S/D 処理により SARS-CoV の不活化が可能であった。
オクタン酸処理はウイルス不活化には不十分であった。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 本研究によって，SARS-CoV のエンベロープ構造を考慮した，S/D 処理，UVC 照射，および 60℃の加熱処理という通常のウイルス不活化によって，SARS-CoV が短時間で不活化されることが確認された。 | 血漿分画製剤の製造工程におけるウイルス除去工程で，SARS-CoV は不活化されることが確認されていることから，現時点で新たな安全対策上の措置を講じる必要はないと考える。 |

**使用上の注意記載状況・その他参考事項等**

BYL-2007-0245

# Evaluation of inactivation methods for severe acute respiratory syndrome coronavirus in noncellular blood products

*Miriam E.R. Darnell and Deborah R. Taylor*

**BACKGROUND:** Severe acute respiratory syndrome coronavirus (SARS-CoV) has been detected in the blood of infected individuals, which may have the potential to contaminate donated blood and plasma-derived products in the event of a future outbreak. Effective methods for inactivating the SARS-CoV in protein solutions are described in this report.

**STUDY DESIGN AND METHODS:** Heat, ultraviolet (UV) irradiation, octanoic acid, and solvent/detergent (S/D) methods were tested individually for their ability to inactivate SARS-CoV in protein solutions appropriately mimicking blood-derived products. Treated samples were tested for inactivation in a tissue culture growth assay.

**RESULTS:** Viral inactivation by heat treatment at 60°C required 15 to 30 minutes to inactivate the SARS-CoV. UVC efficiently inactivated SARS-CoV in 40 minutes, whereas UVA required the addition of psoralen to enhance inactivation of the virus. The presence of bovine serum albumin limited the ability of UVC and UVA to inactivate SARS-CoV and octanoic acid treatment does not reduce the infectivity of SARS-CoV–spiked protein solutions. S/D treatment required 2, 4, and up to 24 hours for Triton X-100, Tween 80, and sodium cholate inactivation, respectively.

**CONCLUSION:** Heat, UVC irradiation, and S/D treatments effectively inactivate SARS-CoV, whereas octanoic acid treatment is insufficient for inactivation of the virus.

Coronaviruses (CoV) are the agents of respiratory and enteric disease in a variety of animals. The life-threatening severe acute respiratory syndrome (SARS) is caused by the recently described coronavirus, SARS-CoV.[1-3] According to the World Health Organization, 8098 people were diagnosed with SARS and 774 people died of this disease during the initial outbreak in 2002 to 2003.

SARS-CoV was detected by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assays in serum from infected patients.[4] Although most, if not all, viremic individuals would be expected to be symptomatic, there is a chance that some fraction of individuals early in infection is asymptomatic and could transmit the virus if they donated blood.

It has been shown that heat or ultraviolet (UV) irradiation of the SARS-CoV in culture medium eliminates viral infectivity.[5] We previously determined that acidic and basic conditions, and formalin and glutaraldehyde, inactivate SARS-CoV in culture medium.[6] Many of the viral inactivation conditions established for SARS-CoV in culture medium, however, are not appropriate for human blood and blood products. Viral inactivation methods used for blood products need to be safe for injection and must not compromise the stability of the proteins.

**ABBREVIATIONS:** CPE = cytopathic effect; HSV-1 = herpes simplex virus-1; RSV = respiratory syncytial virus; SARS-CoV = severe acute respiratory syndrome coronavirus; $TCID_{50}$ = 50 percent tissue culture infectious dose assay; TNBP = tri-(*n*-butyl) phosphate.

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## MATERIALS AND METHODS

### Virus and cells

The Urbani strain of SARS-CoV was grown in African green monkey kidney cells (Vero E6) as described previously.[6] Briefly, Vero E6 cells SARS-CoV (Urbani strain, a gift of L.J. Anderson and T.G. Ksiazek, Centers for Disease Control and Prevention, Atlanta, GA) at $10^{6.33}$ 50 percent tissue culture infectious dose assay ($TCID_{50}$) per mL in Dulbecco's modified Eagle's medium (DMEM; Biosource International, Rockville, MD). After 1 hour at 25°C, DMEM with supplements (10% fetal bovine serum, 2 mmol/L/mL L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 0.5 µg/mL fungizone; Biosource International) was added to the flasks, and the infected cells were incubated at 37°C for 3 days. Supernatants were collected, pooled, clarified by centrifugation, and stored at –70°C for use as the viral stock. The viral stock was filtered through a 0.22-µm filter unit (Millex-GS, Millipore Corp., Bedford, MA) and diluted 10-fold in bovine serum albumin (BSA; Sigma-Aldrich Inc., St. Louis, MO) solutions, phosphate-buffered saline (PBS; Biosource International), or DMEM without supplements for inactivation. Respiratory syncytial virus (RSV) strain A2 was provided by J.A. Beeler (CBER, FDA). Herpes simplex virus-1 (HSV-1) strain 17+ was provided by A.S. Bertke of the FDA. Personnel wore powered air-purifying respirators (3M, St. Paul, MN) and performed all work with infectious virus inside a biosafety cabinet, within a Biosafety Level 3 containment facility.

### Quantitation of viral titers

Viral titers were determined by observing infected Vero E6 monolayers in 24- and 96-well plates with a $TCID_{50}$. Ten-fold serial dilutions of viral samples were incubated at 37°C for 4 days, and then examined for cytopathic effect (CPE) in infected cells as described by Ksiazek and coworkers.[2] Briefly, CPE of SARS-CoV–infected Vero E6 cells was determined by observing rounded, detached cells in close association to each other. An absence of CPE in the Vero E6 cells indicated a loss of infectivity and was evidence of inactivation of the SARS-CoV samples. As the first dilution of viral sample inoculated onto the Vero cells was a 1-in-10 dilution, the limit of viral detection for this assay was 1 log $TCID_{50}$ per mL. If the amount of viral particles was below the limit of detection, then a theoretical, yet low content of viruses may be considered.

### Heat treatment of virus

Virus was diluted in BSA-PBS solutions or PBS, and samples were incubated in a water bath at 56, 60, or 65°C and removed at time points up to 120 minutes. After heat treatment, samples were immediately frozen at –70°C for further analysis by $TCID_{50}$ assay with CPE as an endpoint.

### UV light treatment

UV light treatment was performed with SARS-CoV diluted in BSA-PBS, PBS, or DMEM. Psoralen (Sigma-Aldrich Inc.) was dissolved in 100 percent ethanol to make a stock of 15 mmol per L. SARS-CoV was incubated in a final concentration of 15 µmol per L psoralen for 30 minutes at room temperature and kept in the dark before exposure to UV. Virus samples were placed in 24-well plates (Corning Inc., Corning, NY) and the volume depth at the center of the well was 1 cm. The UV light source (Spectronics Corporation, Westbury, NY) was placed above the plate such that the distance from the light to the bottom of the wells containing the virus samples was 3 cm. At a distance of 3 cm, our UVC light source (254 nm) emitted 4016 µW per $cm^2$ (where $\mu W = 10^{-6}$ J/sec) and the UVA light source (365 nm) emitted 2133 µW per $cm^2$, as measured by radiometric analysis (Spectronics Corporation). After exposure to UV light, virus samples were immediately frozen at –70°C for further analysis by $TCID_{50}$ assay once all of the sampling was completed.

### Octanoic acid treatment

SARS-CoV was diluted in PBS or BSA-PBS. Some of the BSA solutions (pH 6.9) were adjusted with hydrochloric acid (Sigma-Aldrich Inc.) to achieve a pH value of 4.5 or 5.5. RSV and HSV-1 were diluted in DMEM that was similarly adjusted to pH 4.5. Sodium octanoate (stock concentration of 200 mmol/L or 2 mol/L) was added to the samples to achieve a final concentration of 20 or 40 mmol per L. Samples were treated at room temperature or in a 30°C water bath. After a timed incubation, SARS-CoV samples were immediately frozen at –70°C for further analysis by $TCID_{50}$ assay with CPE as the endpoint. RSV and HSV-1 samples were immediately analyzed by $TCID_{50}$ assay.

### Solvent/detergent treatment

Virus was diluted in BSA-PBS protein solutions or PBS. Solvent/detergent (S/D) solutions (20× stock) were added to each sample of virus in BSA-PBS or in PBS to achieve a final concentration of nonvolatile solvent (0.3% v/v), specifically, tri-(*n*-butyl) phosphate (TNBP; Aeros Organics, Pittsburgh, PA) and either 1 percent Tween 80 (Sigma-Aldrich Inc.), 1 percent Triton X-100 (Sigma-Aldrich Inc.), or 0.2 percent sodium cholate (Sigma-Aldrich Inc.). Before the addition of the S/D solution and at the time points 2, 4, 6, and 24 hours after the addition of the S/D solution, an aliquot was removed from each of the samples and diluted 10-fold (Tween 80 and sodium cholate) or 100-fold (Triton X-100) in DMEM. This dilution was necessary to stop the inactivation reaction and to negate the cytotoxic effects of the S/D on the Vero cells during the titration analysis.[7] The dilution step was considered during the

final calculation. Immediately after this dilution in DMEM, the aliquots were frozen at –70°C and then analyzed by $TCID_{50}$ assay with CPE as an endpoint.

### Statistical analysis

Data are presented as the geometric mean of three or more samples ± standard deviation.

## RESULTS

To evaluate the efficacy of treatments used to inactivate viruses in human blood products, we examined SARS-CoV survival under current protocols. To validate these techniques, BSA protein solutions were spiked with SARS-CoV, whereas PBS or DMEM were spiked with the virus and used as a control. After treatment to inactivate SARS-CoV, these virus-spiked solutions were tested for infectivity in a $TCID_{50}$ assay with a limit of viral detection of not greater than 1 log $TCID_{50}$ per mL.

### Heat inactivation of SARS-CoV

Heat may inactivate viruses by denaturing the secondary structures of proteins and thereby may alter the conformation of virion proteins involved in attachment and replication within a host cell.[8,9] To test the ability of heat to inactivate SARS-CoV, we incubated normal human serum spiked with virus at two temperatures (56 and 65°C) for increasing periods of time. After 20 minutes at 56°C, SARS-CoV was inactivated to the level of the detection limit for the assay (Fig. 1A). Human serum treated at 65°C required only 10 minutes to achieve viral inactivation to the detection limit of the assay (Fig. 1A).

To test the ability of heat to inactivate SARS-CoV in solutions with higher protein concentrations, we incubated BSA solutions or PBS spiked with virus at 60°C for increasing periods of time. After 15 minutes at 60°C, inactivation of the virus in the PBS, 10 percent BSA, and 16 percent BSA solutions was observed (Fig. 1B). Although the virus in 10 percent BSA appeared to be inactivated in 15 minutes, there was a small, yet detectable amount of virus present in one of the triplicate 25-minute samples (Fig. 1B). This outlier may have arisen due to experimental error or the virus may have aggregated thus making it more resistant to inactivation. By 30 minutes, SARS-CoV was inactivated in the 10 percent BSA solution and was no longer detectable for the duration of the experiment (120 min). Fifteen minutes at 60°C significantly decreased, to near the limit of detection of the assay, the titer of SARS-CoV in the 25 percent BSA protein solution (Fig. 1B). Unfortunately, at 20 minutes, the 25 percent BSA solutions were denatured and rendered them inappropriate for virus titer testing. These results suggest that SARS-CoV inactivation by pasteurization may be effective; however, the rate of inactivation may be dependent upon protein concentration.

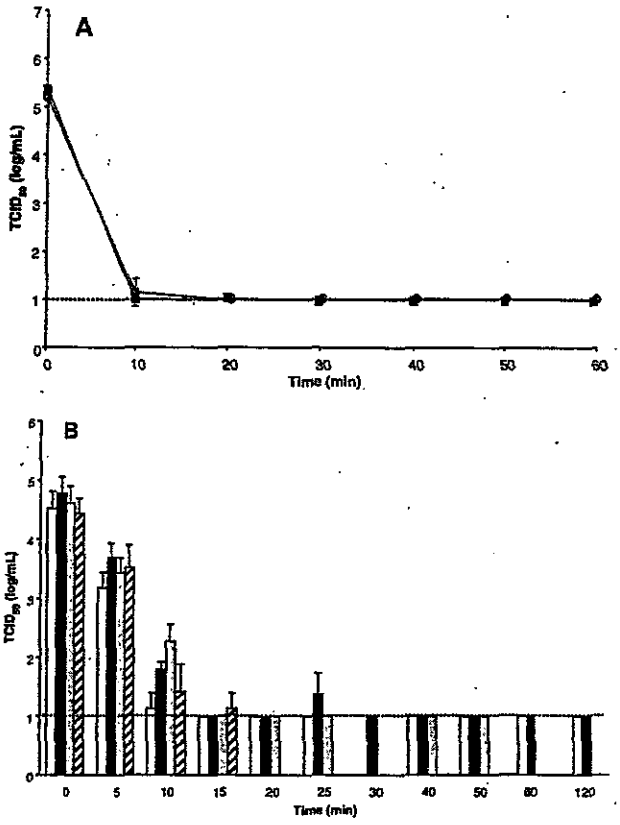


**Fig. 1. Inactivation of SARS-CoV by heat treatment of (A) human serum and (B) BSA solutions. SARS-CoV was diluted 1:10 in human serum, PBS, or BSA solutions. The virus/diluent mixtures were incubated in a water bath at (A) 56°C (○) and 65°C (●) and (B) 60°C, and samples were removed at designated time points. Samples were frozen and analyzed simultaneously by $TCID_{50}$ assay. The broken line shows the limit of detection for the assay. (□) PBS; (■) 10 percent BSA; (▤) 16 percent BSA; (▨) 25 percent BSA.**

### Effect of UV radiation on SARS-CoV

The continuum of UV light is divided into three classifications: UVC (200-280 nm), UVB (280-320 nm), and UVA (320-400 nm). UVC and UVB light causes pyrimidine dimers,[10] whereas UVA may cause RNA and DNA strand breaks.[11] The effect of UV irradiation on SARS-CoV infectivity was examined by spiking solutions of BSA or DMEM with SARS-CoV in 24-well tissue culture plates on ice. The samples were exposed to UV light for varying lengths of time and tested for infectivity. As expected from our previous results,[6] UVC treatment significantly decreased the virus titer after 20 minutes and inactivated SARS-CoV in PBS to the limit of detection by 40 minutes (Fig. 2A). UVC

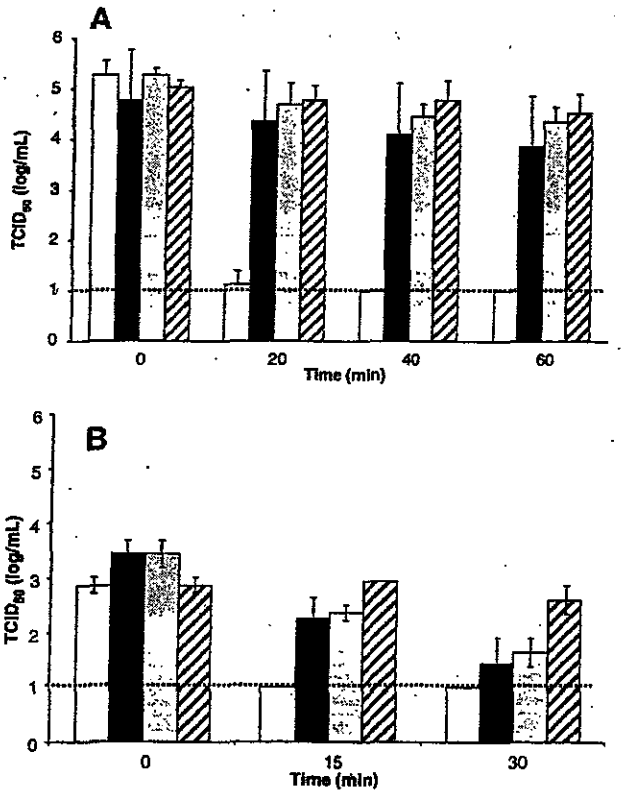


Fig. 2. Inactivation of SARS-CoV by ultraviolet light. (A) Virus was incubated in PBS, with and without BSA, and exposed to UVC light for the indicated times. (□) PBS; (■) 10 percent BSA; (▨) 16 percent BSA; (▨) 25 percent BSA. (B) Virus was mixed into DMEM, with and without 10 percent BSA. (□) DMEM, psoralen; (■) 10 percent BSA, psoralen; (▨) DMEM, no psoralen; (▨) 10 BSA, no psoralen. Psoralen was added to half of the viral samples. All viral samples were analyzed for $TCID_{50}$ after exposure to UVA. The broken line shows the limit of detection for the assay.

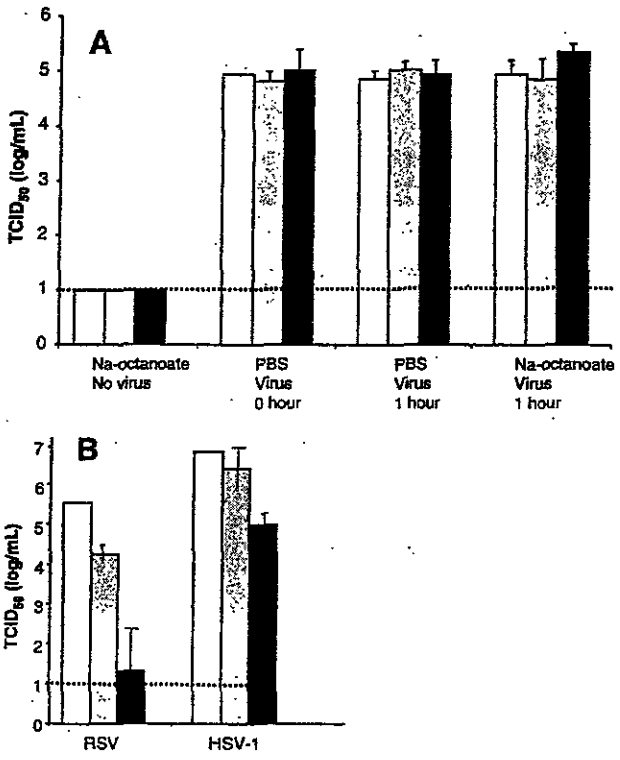


Fig. 3. Octanoic acid treatment of virus. (A) SARS-CoV was diluted into 10 percent (□), 16 percent (□), and 25 percent (■) BSA solutions with octanoic acid (20 mmol/L) or PBS. (B) Octanoic acid (40 mmol/L) was added to virus stocks (RSV or HSV-1) in DMEM and then analyzed for infectivity by $TCID_{50}$ assay. The broken line shows the limit of detection for the assay. (□) Time 0; (□) 1 hour; (■) 24 hours.

treatment, however, did not inactivate the virus in BSA protein solutions, and the virus was still infectious even after 60 minutes of treatment (Fig. 2A). Additionally, lower concentrations of protein resulted in some inactivation after exposure to UVC. We determined that some inactivation of SARS-CoV occurred in 2 and 5 percent BSA protein solutions but to a lesser extent than when no protein was present (data not shown). This suggests that there is a concentration-dependent protective effect of BSA not present in PBS alone.

UVA treatment to inactivate viruses in platelet (PLT) concentrates is combined with psoralen compounds to increase the effectiveness of the method.[12] We determined that UVA treatment of SARS-CoV in DMEM decreased the infectivity by 100-fold after 30 minutes, whereas the addition of psoralen inactivated the virus to the limit of detection after only 15 minutes (Fig. 2B). Although UVA treatment of SARS-CoV in 10 percent BSA resulted in no inactivation after 30 minutes, the addition of psoralen brought about a 100-fold decrease in infectivity during the same amount of time, yet did not result in complete inactivation of the virus (Fig. 2B). These data suggest that the addition of psoralen increases the effectiveness of UVA treatment regardless of the presence of BSA protein, and the presence of BSA protein limits the effectiveness of UVA inactivation of SARS-CoV.

## Effect of octanoic acid on SARS-CoV

Octanoic acid is commonly used to inactivate lipid-enveloped viruses during the production of human blood products.[13] To determine the effect of octanoic acid on SARS-CoV, virus-spiked BSA solutions were incubated with octanoic acid for 1 hour at pH 6.9 and at room temperature. Octanoic acid had no apparent effect on the infectivity of SARS-CoV (Fig. 3A). We also determined that 1 hour incubation of SARS-CoV in BSA solutions with octanoic acid at pH 4.5 or 5.5 did not inactivate the virus

(data not shown). Changing the conditions by lengthening the time of incubation to 24 hours, increasing the concentration of octanoic acid from 20 to 40 mmol per L, and increasing the temperature to 30°C, while maintaining the acidic pH of 4.5, also did not inactivate SARS-CoV (data not shown). When RSV was exposed to 40 mmol per L octanoic acid at 30°C with a pH value of 4.5, however, the titer was significantly decreased after 24 hours when compared to untreated virus (Fig. 3B). The octanoic acid treatment of HSV-1 for 24 hours did not reduce the titer nearly as effectively as it did for RSV (Fig. 3B). Although this method is effective for the inactivation of many lipid enveloped viruses, such as RSV and to a lesser extent, HSV-1, it does not appear to inactivate SARS-CoV under any of the conditions described.

### S/D treatment of human serum spiked with SARS-CoV

S/D treatment is a standard method used to inactivate viruses in human blood products.[7,14] SARS-CoV–spiked solutions of BSA and PBS were treated with S/D, incubated at room temperature, and sampled periodically over 24 hours. The SARS-CoV–spiked samples treated with TNBP/Triton X-100 were inactivated to the limit of detection by 2 hours, regardless of whether the virus was in PBS or in various BSA solutions (Fig. 4A). TNBP/Tween 80 treatments efficiently inactivated SARS-CoV in PBS and in 10 percent BSA within 2 hours; however, 4 hours were required to inactivate the virus to the limit of detection in 16 and 25 percent BSA protein solutions (Fig. 4B). SARS-CoV in PBS was inactivated by treatment with TNBP/sodium cholate after 2 hours; however, 24 hours were required to inactivate the virus to the limit of detection in 10 percent BSA (Fig. 4C). The virus titer in 16 and 25 percent BSA protein solutions was only decreased slightly after TNBP/sodium cholate treatment and was still detectable after 24 hours. These results suggest that the effectiveness of SARS-CoV inactivation by S/D treatment is variable, depending upon the specific method used, duration of treatment, and the protein content of the solution.

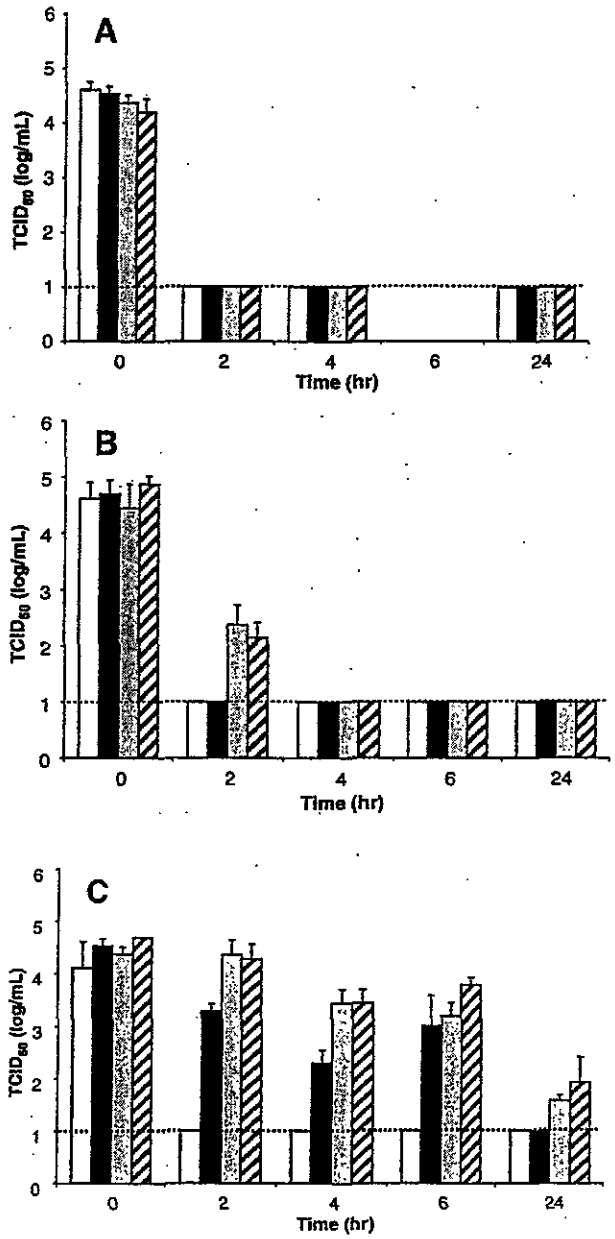


**Fig. 4. S/D treatment of SARS-CoV. Virus was diluted in PBS or BSA solutions. The S/D concentrate (20×) was added and samples were removed at designated time points and frozen immediately. The detergents used were (A) Triton X-100, (B) Tween 80, and (C) sodium cholate. The broken line shows the limit of detection for the $TCID_{50}$ assay. (□) PBS; (■) 10 percent BSA; (▨) 16 percent BSA; (▨) 25 percent BSA.**

## DISCUSSION

Transmission of SARS-CoV occurs predominantly by respiratory droplets over a relatively close distance.[15] Although blood transfusions and administration of blood products have not been implicated in SARS-CoV transmission,[16] low to moderate levels of viremia during the clinical phase of the illness have been shown.[1] Nie and associates[17] have shown that neutralizing antibodies to SARS-CoV can be detected as early as 5 to 10 days after onset of symptoms. With an immunofluorescent antibody assay, Hsueh and associates[18] have detected immunoglobulin (Ig)G seroconversion by Day 10 and IgM and IgA seroconversion on Day 11. Wang and coworkers[19] determined that SARS-CoV RNA was detectable by quantitative real-time RT-PCR assay as early as 2 days after onset of SARS symptoms in 50 percent of the patient plasma samples they examined. In 75 percent of SARS patients they found peak

viral RNA titers detected at 5 to 7 days after symptom onset. These data do not exclude the possibility that (in the event of an outbreak) SARS may be present in the blood of asymptomatic but infected individuals at undetectable levels. Owing to the possibility for SARS-CoV transmission via plasma-derived components, current viral inactivation methods need to be evaluated for effectiveness against SARS-CoV.

Pasteurization at 60°C for 10 hours is used to inactivate potential viruses in plasma-derived products.[20] An advantage of this technique is that it is effective against both enveloped and nonenveloped viruses.[20] Stabilizers to protect the protein product, however, may also protect or mask the virus. Neoantigens may be formed from partial denaturation of human serum components that may appear foreign to the immune system.[20] This could be deleterious, because it may result in the onset of autoimmune disease. We determined that SARS-CoV was inactivated by pasteurization at 60°C in less than 1 hour; however, protein concentration affected the time required for inactivation. Previously we examined the ability of heat to inactivate SARS-CoV when in DMEM instead of human serum or BSA solutions. We found that 90 minutes at either 56 or 65°C was necessary to inactivate the virus.[6] Yunoki and associates[21] examined the sensitivity of SARS-CoV to 60°C heat and found that the different protein compositions of the samples tested affected the time necessary, yet all samples were inactivated to the limit of detection by 1 hour. Additionally, Rabenau and coworkers[22] tested heat inactivation of SARS-CoV at 56°C for 30 minutes and determined that the presence of 20 percent fetal calf serum protected the virus. We observed that incubation at 60°C for 30 minutes, however, was sufficient for complete inactivation to the limit of detection. Given that pasteurization of plasma-derived products is usually performed at 60°C for 10 hours,[20] any SARS-CoV present in such products, regardless of protein concentrations and in the presence of stabilizers, should be efficiently inactivated.

Both UVC and UVA, in conjunction with psoralen, can be used to inactivate pathogenic viruses in plasma-derived products. Previously we determined that UVA light alone did not inactivate SARS-CoV in DMEM after 20 minutes.[6] Lin and coworkers[12] added psoralen S-59 to PLT concentrates and exposed them to UVA light. The authors determined that 3 J per $cm^2$ UVA light for 3 to 4 minutes with the addition of 150 µmol per L psoralen was sufficient to inactivate human immunodeficiency virus, duck hepatitis B virus (a model for hepatitis B virus), and bovine viral diarrhea virus (a model for hepatitis C virus) and that neither psoralen, nor UVA light alone, was sufficient to inactivate these viruses. We determined that this also holds true for SARS. The addition of intercalating agents, such as psoralen, may be required to increase the efficiency of UVA viral inactivation and may be appropriate for treatment of PLTs or red cells.

We determined that UVC had no inactivating effect on the virus in 10 to 25 percent BSA solutions. Previously, however, we inactivated SARS-CoV in DMEM[6] suggesting that some characteristic of the BSA protein protected the virus from inactivation. It is possible that BSA interfered with the light waves and prevented them from reaching the virus in the solution. Chin and colleagues[23] inactivated several virus types in plasma and Factor VIII concentrate by circulating the samples through a flat quartz cell that was 0.22 mm thick. Li and coworkers[24] described that UVC light was decreased by $10^5$ at 1 mmol per L depth in a 20 percent (w/v) albumin solution. These data suggest that there is some factor in the BSA protein solutions, which has a protective effect on SARS-CoV, and this effect might be overcome by exposing a very thin layer of the SARS-CoV solution to the UVC light.

Octanoic acid, or caprylate, is a treatment used to inactivate enveloped viruses in human blood products. The mechanism of action is due to the lipophilic nature of the nonionized form of the acid, which allows it to insert itself into the viral membrane and disrupt the lipid envelope and/or the proteins imbedded in the envelope.[25] Johnston and colleagues[26] have shown that caprylate treatment is effective against Sindbis virus and bovine viral diarrhea virus. We determined that SARS-CoV was resistant to inactivation by octanoic acid treatment, even under the conditions of acidic pH (4.5), elevated temperature (30°C), a higher concentration (40 mmol/L) of octanoate, and a longer treatment time (24 hr). Based on the work of Lundblad and Seng,[25] we exposed both RSV and HSV-1 to octanoate as a control. Octanoic acid treatment decreased the titer of RSV, suggesting that our conditions were correct for inactivation. In contrast, Lundblad and Seng[25] effectively inactivated HSV-1 with octanoic acid at similar conditions, but the octanoic acid was removed with an ion-exchange column, and this may account for differences in the end result. Under the conditions tested here, octanoic acid treatment was ineffective for inactivation of SARS-CoV.

S/D methods are not used for inactivation of viruses in cellular blood components because S/D treatment damages cell membranes.[27] The S/D method causes a disruption of the structural integrity of lipid-enveloped viruses.[20] The S/D treatment has the advantage of being highly efficient at inactivating enveloped viruses, while being nondenaturing on proteins, and yielding a high recovery of protein activity.[20] The disadvantages include requiring an extra manufacturing step to remove the S/D agents and the inability to inactivate nonenveloped viruses.[20] In our experiments, both Triton X-100 and Tween 80 inactivated SARS-CoV in BSA solutions. Sodium cholate was ineffective in high protein concentrations

even after 24 hours of treatment. There may be a protective effect by some of the proteins in the BSA solutions, as the SARS-CoV in the higher concentrations of BSA was not inactivated as quickly by Tween 80 or sodium cholate as it was with Triton X-100. Horowitz and associates[28] have shown that the S/D method can very effectively inactivate cytomegalovirus, Sindbis virus, and other pathogenic viruses. The Tween 80 and Triton X-100 methods should be sufficient to inactivate SARS-CoV in plasma for the manufacture of plasma-derived products.

Human plasma–derived components potentially contain transfusion-transmissible viruses. To minimize the risk of infection, the manufacturing process includes two or more inactivation steps. Several methods show promise and should minimize the potential risk of transmission of SARS-CoV from human plasma–derived components.

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# 医薬品　研究報告　調査報告書

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**研究報告の概要**

○高病原性トリインフルエンザA H5N1、最新情報2006
2003年以来2006年11月29日時点で、H5N1型トリインフルエンザウイルス感染患者258名がWHOに報告されている。このうち154名が死亡したが（60%）、高い致死率の低下は認められていない。小児や若年成人に感染者が多く、10歳～29歳の女性の患者発生が顕著である。遺伝的な感受性を示唆する家族内患者集積、無症候性や軽症感染も発生している。ヒトーヒト感染は極めて起こりにくい。
2006年末現在、H5N1型ウイルスは50カ国以上で鳥類での感染が確認されており、うち10カ国では鳥類がヒト患者発生の感染源となっている。一部の国では家禽に感染が定着し、住民への感染リスクが常在する事態に直面している。大規模な家禽のワクチン接種計画が中国とベトナムで進行中である。EUでは、同ウイルスは家禽には感染定着しておらず、2006年春季に少なくとも15カ国で野鳥の感染が確認されたが、ヒト感染症例は発生していない。家禽の感染予防が成功し、感染例は5件のみで迅速に制圧された。散発例の報告が続いていることから、生物学的安全確保対策と早期警報システムを堅持する必要がある。
現在、同ウイルスのヒトへの影響に明確な変化は現われていない。しかし、遺伝子の多様性と連続的な遺伝子変異は、抗ウイルス薬の準備やプレパンデミックワクチンの製造に影響するため、重要な検討課題である。感染が生じた場合には、地域での流行に対する備えや対応が、動物衛生や公衆衛生に関する帰結を決定する上で最も重要となる。効果的なサーベイランス/早期警報および生物学的安全確保を可能とする全国的に組織化された獣医学サービスが必須である。

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 2006年11月29日時点でH5N1型トリインフルエンザウイルス感染患者258名がWHOに報告され、50カ国以上で鳥類での感染が確認されており、うち10カ国では鳥類がヒト患者発生の感染源となっているとの報告である。 | 日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。H5N1型トリインフルエンザは日本でも鶏から検出されており、新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。 |

64

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1. Highly Pathogenic Avian Influenza A/H5N1 – update and overview of 2006
2. Soft tissue infections in Belgian rugby players due to *Streptococcus pyogenes emm* type 81
3. Epidemic intelligence during mass gatherings
4. Cooperation between animal and human health sectors is key to the detection, surveillance, and control of emerging disease: IMED 2007 meeting in Vienna, February 2007

**This is the last weekly release of 2006. Eurosurveillance will return on Thursday 4 January 2007. From the Eurosurveillance editorial team in England, France and Sweden, we wish all our readers a merry Christmas, a happy New Year, and a peaceful holiday.**

## Highly Pathogenic Avian Influenza A/H5N1 – update and overview of 2006

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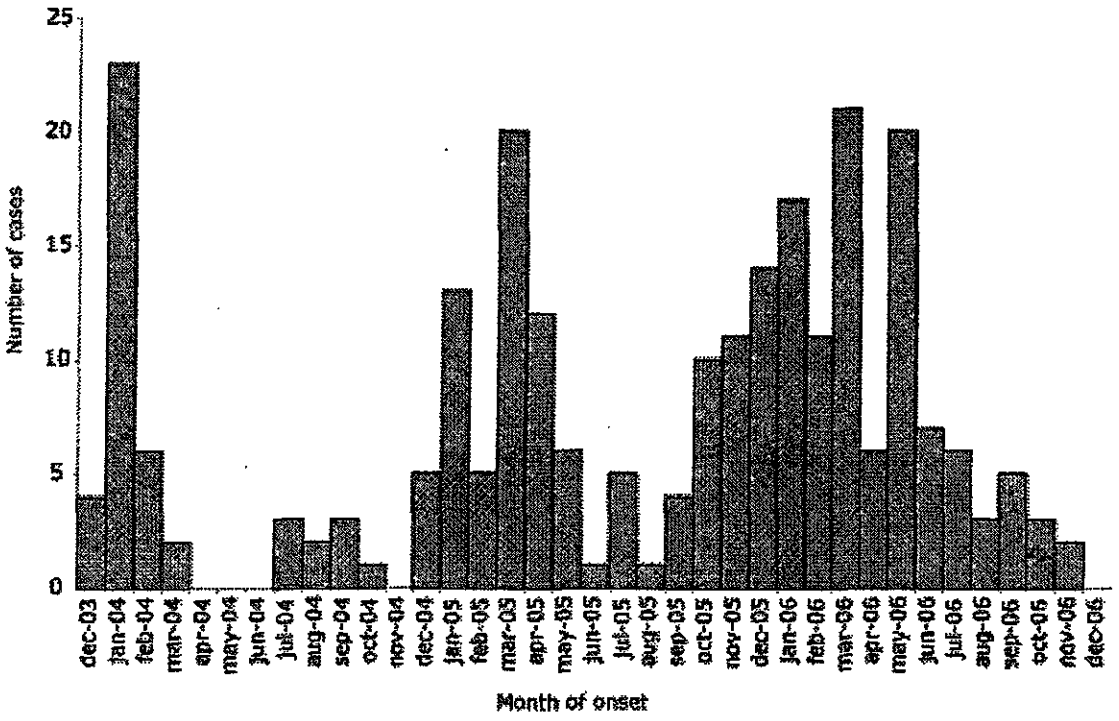
### Avian influenza 2006: human situation

As of 29 November 2006, 258 human H5N1 infections that meet its strict laboratory criteria have been reported to the World Health Organization (WHO) since reporting began for 2003. Of these 258 cases, 154 patients have died (60%) and there has been no decline in that high mortality rate over time [1,2]. There has been a disproportionate concentration of infections in children and young adults, even allowing for the relatively young populations in the ten countries where human infections have occurred, and there is an over-representation of females among patients aged 10-29 years [2]. This is thought to be related to the fact that it is usually young people and women who look after domestic poultry. There is some evidence of familial clustering which may suggest a genetic susceptibility [3,4]. Asymptomatic and mild infections do occur but appear to be very rare, although more sero-epidemiology around confirmed cases is needed to confirm this impression [3-6]. In the second half of 2006, there was a steep decline in the number of case reports, although similar declines occurred in 2004 and 2005, but were then followed by resurgences (Figure 1) [1,2].Critically, human to human transmission, as indicated by cluster size, is still extremely inefficient, as it was a decade ago when the first human to human transmission took place in Hong Kong [3-5].

**Figure 1.** No. of confirmed human cases of H5N1 infection reported to WHO by month of onset, 1 December 2003 – 11 December 2006




Based on reports to WHO published at http://www.who.int/csr/don/archive/disease/en/index.html using method used by WHO and descri[illegible]in Reference 2

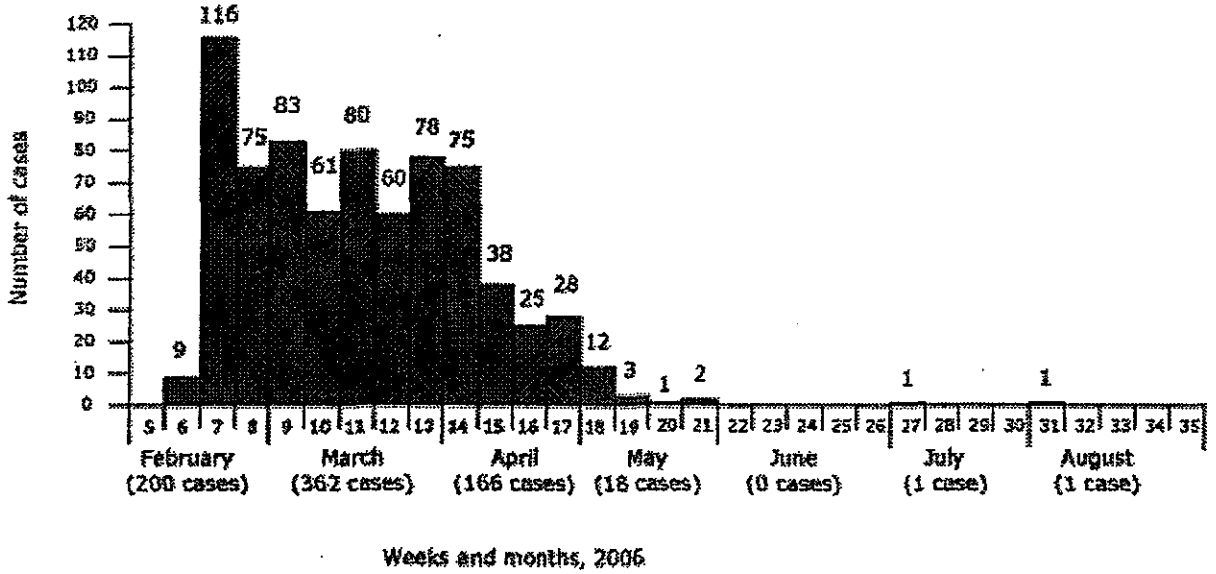
**Animals still source of human infections**

In 2003, highly pathogenic avian influenza viruses type A/H5N1 (Asian strain) re-emerged and spread rapidly, infecting poultry and some humans in a number of southeast Asian countries, particularly Vietnam, Thailand, Cambodia and Indonesia [7]. The mechanism for this spread remains unclear although it is suspected that it was as much related to trade of poultry and poultry products as the movements of wild birds. An exceptional multi-species epizootic at Qinghai Lake in northwest China in May 2005 seemed to demonstrate a role of wild birds in the spread of the viruses beyond Asia [8]. From Qinghai, the virus spread to Central Asia, Europe and some African countries with human cases reported in Turkey, Iraq, Azerbaijan, Djibouti and Egypt [1,2,7]. Now, at the end of 2006, the virus has been confirmed in birds in over 50 countries, with birds (almost entirely domestic poultry) being the source of human infections in ten of these [2,7,9].

Some countries are facing up to endemic infection in their national poultry flock and consequent ongoing risks to humans with domestic poultry, while others are barely affected. At a recent world conference on avian influenza and pandemic preparedness [footnote], field reports on efforts to control avian influenza were presented by national and international authorities. There is evidence that H5N1 viruses have now become entrenched in backyard poultry in Indonesia, and perhaps also Egypt [10,11]. Large scale programmes of poultry immunisation have been underway in China and Vietnam where, since 2005 and until an outbreak in the Mekong Delta this week in Vietnam [12], poultry outbreaks had stopped being reported [9]. The scale of immunisation in China, with potentially 14 billion poultry needing to be vaccinated twice annually (in spring and autumn), is the largest immunisation programme against avian influenza ever attempted anywhere in the world.

In the European Union (EU), the virus has not become established in poultry nor have there been there any human infections even though the virus was found in wild birds in at least fifteen countries in the spring of 2006 (Figure 2). Some cats and a pine marten that fed on infected birds were also infected [13]. The bird movements to the EU may have been exceptional following an unusually cold spell of weather in Russia and Central Asia in early 2006. After the spring wave, there have only been confirmation of sporadic H5N1 infections in birds in Spain and Germany (Figure 2).

**Figure 2.** Highly pathogenic avian influenza cases in wild birds in the EU member states notified to the European Commission in 2006.




Based on reports through reports to the European Commission Animal Disease Notification System (ADNS) http://ec.europa.eu/food/animal/diseases/adns/adns_en.htm as cited in Reference 14. Cases are almost entirely due to A/H5N1.

The successful protection of domestic birds in EU countries was primarily due the robust and consistent application of veterinary measures directed under EU legislation. As a consequence, only five poultry outbreaks occurred in the EU and these were rapidly contained [14,15]. However, the continuing sporadic reports demonstrate that the virus may still sometimes be present and therefore, routine biosecurity measures and early warning systems cannot be relaxed. There were major outbreaks of infection in wild birds and domestic poultry in the Danube delta in 2005 and 2006, and the Romanian authorities successfully contained these. There will be an additional challenge for EU authorities if it occurs here again after Romania joins the European Union next month.

**Continuing evolution of the viruses**

There remains the risk of emergence of a human pandemic strain through either mutation of the H5N1 virus or incorporation of part of its genome, through recombination, into a human influenza virus [7,16]. As well as extending their range geographically the H5N1 viruses have diversified genetically into clades and sub-clades. Clade 1 dominated in 2003-2004, then clade 2 became more important. Clade 2 has subsequently developed into three distinct sub-clades [7,17,18]. The balance between the types of virus continues to change, for reasons that are not clear. For example, since 2005, the Fujian-like virus (clade 2, sub-clade 3) has become the dominant type found in surveillance of market poultry across southern China [17]. Fortunately, despite genetic changes, there has been no evidence of significant change in the viruses' effects on humans. The genetic differences and the fact that the virus is continuing to change are, however, important considerations since the clades have different antiviral resistance profiles and continuing genetic change will alter the necessary composition of human H5N1 vaccines referred to as 'pre-pandemic vaccines' [7,18]. Two countries have already committed to purchasing these vaccines and others are considering to do so, although it is by no means clear that an H5 based pandemic is inevitable[7,16].

**Discussion**

There are many important unknown factors relating to the spread of H5N1, including the current distribution of the viruses. The pattern of H5N1 infection in Africa remains elusive because surveillance is especially weak there, apart from Egypt and some parts of Nigeria [11,19]. The picture is also incomplete in eastern Asia - following two human cases in summer 2006, the situation has improved in Thailand, but the risk remains [20]

A good picture of the zoonotic situation in China is currently not available and it is also still unclear whether the H5N1 vaccination programmes in China and Vietnam have been successful in eliminating or just reducing the level of infection in poultry, and whether low levels of circulating viruses pose a significant human risk [7,21] One negative consequence of any success of vaccination programmes is that surveillance for sporadic human cases is made more difficult, since now, when atypical pneumonias occur, there is rarely the marker of local poultry deaths to inform decisions on whether to test the patient for H5N1 virus.

The relative role of the commercial movement of animals and wild birds in the international spread and local distribution of H5N1 viruses remains controversial. However, it is local preparedness and response that are most crucial in determining the outcome in terms of domestic animal and human health when countries are challenged by the virus. Nationally organised veterinary services, which would enable effective surveillance/early warning and biosecurity systems, are crucial so that authorities can respond promptly when

infections are first suspected in either birds or humans. Where biosecurity is poor and veterinary services ineffective, viruses can become endemic and the situation can be complicated by the virus cycling between poultry and wild birds [10,11,17].

One challenge developing countries face is a lack of financial support for the veterinary services and biosecurity measures, even though avian influenza has demonstrated that it is truly an international problem. There has been some progress towards a solution for the financial issues by the involvement of the World Bank, the European Commission and the United Nations System Influenza Coordinator (http://www.undg.org/content.cfm?id=1482), which have mobilised and released donations that had been pledged by national and international donors [22].

The data indicate that H5N1 avian viruses remain poorly adapted to humans. With a high enough viral challenge and perhaps some genetic host susceptibility the viruses can infect humans, in which case they are then often lethally pathogenic, although they are still unable to transmit efficiently between humans [2-5,16]. The H5N1 viruses have been around for nearly a decade and it might be tempting to conclude that if they were going to proceed to form or contribute to a pandemic strain, they would have done so by now. However, it should be remembered that it is thought that the avian influenza virus which contributed to the 1918-19 'Spanish Influenza' H1N1 pandemic strain had been around for some years before it became part of a virus that could efficiently transmit between humans and so be a successful pandemic strain [23].

Apart from the threat from H5N1 there are still many issues around influenza pandemic preparedness (irrespective of the virus type) which need urgent attention. One key area is how authorities in developing countries should best focus their efforts with preparedness, given often very limited resources and many more immediate competing priorities. So far, most discussion, ideas and research have been more suited to settings in better resourced nations. This area needs a multi-sector approach as medical services will not have the most to offer in poorer countries when it comes to preparing for a pandemic. It is hoped that the next world meeting, planned for New Delhi in late 2007 (and intervening technical meetings), will provide opportunities to tackle preparedness in developing nations as well as dealing with avian influenza.

*Footnote. The Bamako conference organised by the African Union, the Interafrican Bureau for Animal Resources and the European Union. International Conference on Avian and Human Pandemic Influenza, (Ministerial Meeting and Pledging Conference) 6-8 December 2006, Bamako.*
*Documentation and presentations at the conference are viewable at : http://www.avianinfluenzaconference4.org/index.php?id=61*

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back to top

別紙様式第2-1

No. 15

# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2006. 12. 5 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | ProMED 20061201-3394, 2006 Dec 1. 情報源:CIDRAP News, 2006 Nov 29. | 公表国 WHO |
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| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | |

**研究報告の概要**

○WHOがH5N1ヒト症例の診断ガイドラインを発表

WHOは、ウイルスにより光を当て、パンデミック株への変異の検出を容易にするために、H5N1鳥インフルエンザのヒト症例診断のためのガイドラインを発表した。

14ページのガイドラインは、患者の問診、周辺で他の症例を捜索することによる接触歴の調査、ヒトーヒト感染の何らかの徴候を発見するためのデータのふるいわけなど、各症例の徹底的な調査を求めている。ガイドラインでは、臨床検査の結果が出る前に疑い症例の調査を行うことを要請している。

症例診断の目的は、(1)H5N1の診断確定(2)早期発見、治療、予防による疾病と死亡の削減(3)感染源の早期特定と予防策実施による拡大防止(4)ヒトーヒト感染がより効率的になってきているかどうかの確定(5)各症例の疫学的、臨床的、ウイルス学的特徴の識別(6)臨床医、調査担当者、行政の間で常に情報を交換し、適切な対応を可能とすることである。

患者の接触歴の調査に当たって、WHOは患者は発症前1週間、治癒後2週間感染能を持つとみなすべきであるとしている。接触歴調査に加えて、調査担当者は家々の訪問や、医療機関や開業医、研究所への電話調査を行い、他のH5N1可能性例を積極的に探すべきであると勧告している。検査については、「商用の迅速診断検査の性能は不明であり、サブタイプの判別は出来ないため確認検査が必要である」としている。

ガイダンスでは、ウイルスのヒト間の伝播性における変化の手がかり、症例のクラスターを評価する上での注意点についても述べている。また、感染地域内の職業上リスクがある人の血清学検査、感染リスク要因を評価するための症例対照研究などの補足的研究の実施を提案している。

| 報告企業の意見 | 今後の対応 |
|---|---|
| WHOがH5N1型トリインフルエンザのヒト症例の診断ガイドラインを発表したとの報告である。 | H5N1型トリインフルエンザは日本でも鶏から検出されており、新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。 |

**使用上の注意記載状況・その他参考事項等**

合成血「日赤」
照射合成血「日赤」
合成血-LR「日赤」
照射合成血-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

57

**Archive Number** 20061201.3394
**Published Date** 01-DEC-2006
**Subject** PRO/AH> Avian influenza, human (185): WHO investigation guidelines

AVIAN INFLUENZA, HUMAN (185): WHO INVESTIGATION GUIDELINES
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Thu 30 Nov 2006
From: Brent Barrett <salbrent@sbcglobal.net>
Source: CIDRAP News, Wed 29 Nov 2006 [edited]
<http://www.cidrap.umn.edu/cidrap/content/influenza/avianflu/news/nov2906inves

The World Health Organization (WHO) this week released guidelines for investigating human cases of H5N1 avian influenza in an effort to shed more light on the virus and improve the chances of detecting changes that could turn it into a pandemic strain.

The 14-page document calls for a thorough probe of each case, from interviewing the patient and searching for contacts through hunting for other cases nearby and sifting data for any signs of human-to-human transmission. It calls for investigating suspected cases before laboratory test results are available.

The new guidance comes less than a month after the WHO issued a report on how much remains unknown about the H5N1 virus. Written by a group of leading flu experts, that report, issued Nov 2, said H5N1 illness is fundamentally different from ordinary flu in its severity and range of manifestations. Among other things, the panel called for research to determine why children and young adults seem especially susceptible and whether genetic factors increase the risk of infection or transmission among blood relatives.

The introduction to the new guidance says, "The document reflects and incorporates the practical field experience gained by investigators working at international, national, and sub-national levels during investigations of A(H5N1)."

The intention is that local health agencies will use the guidelines to build their own investigative plans and procedures, the WHO says. The purposes of case investigation, according to the guidelines, include:

(1) Confirming the diagnosis of H5N1;
(2) Reducing illness and death by rapidly identifying cases and starting appropriate treatment and precautions;
(3) Reducing further spread by identifying possible sources of exposure and implementing prevention and control measures;
(4) Determining if human-to-human transmission is becoming more efficient;
(5) Identifying the key epidemiologic, clinical, and virologic characteristics of each case;
(6) Ensuring timely exchange of information among clinicians, health investigators, and government officials to facilitate appropriate responses.

In advice on searching for patients' contacts, the WHO says investigators should assume that patients are infectious for 1 week before onset of illness and 2 weeks afterward, even though the

infectious period for treatment, depending on their risk of exposure, as defined in other WHO guidance.

Besides tracing contacts, investigators should search actively for other possible H5N1 cases by going from house to house and possibly conducting telephone surveys of healthcare facilities, practitioners, and labs, the WHO advises.

The agency recommends against using rapid diagnostic tests for the H5N1 virus. "The diagnostic accuracy of commercially available rapid tests is unknown, and if the test result is positive, differentiation between influenza A subtypes is not possible and confirmatory tests must be done," the document says.

In its 2 Nov 2006 report, the WHO said a rapid, reliable diagnostic test for H5N1 infection was urgently needed.

The guidance document discusses potential clues to a change in the virus's human transmissibility and what to look for in evaluating clusters of cases. It also suggests doing complementary studies, such as seroprevalence surveys of people with possible occupational risk in affected areas and case-control studies to evaluate risk factors for infection.

[As of 30 Nov 2006, the full report: "WHO guidelines for investigation of human cases of avian influenza A(H5N1) - October 2006" at
<http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_6
is currently under revision. - Mod.CP]

--
Brent Barrett
<salbrent@sbcglobal.net>

[These guidelines reflect concern that human cases of H5N1 virus may be escaping detection because of some difficulties encountered in diagnosis, as expressed recently in 2 papers published in the New England Journal of Medicine (see ProMED-mail post entitled: "Avian influenza, human (181): Indonesia, Turkey 20061124.3337" for detailed discussion).

See also Influenza research at the human and animal interface. Report of a WHO working group. Geneva, Switzerland. 21-22 Sep 2006. WHO/CDS/EPR/GIP/2006.3
<http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_
- Mod.CP]

[see also:
Avian influenza, human (181): Indonesia, Turkey 20061124.3337
Avian influenza, human (178): receptor mutations 20061116.3279
Avian influenza, human (175): new sub-lineage 20061108.3201
Avian influenza, human (173): new sub-lineage 20061102.3144
Avian influenza, human (172): genetic disposition 20061102.3140
Avian influenza, human (140): atypical infections 20060905.2522
Avian influenza, human (133): WHO case definitions 20060830.2467
Avian influenza, human (91): WHO epidemiology analysis 20060630.1812]
.....cp/pg/lm

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INFLUENZA >> AVIAN INFLUENZA >> NEWS >>

# WHO urges careful investigation of H5N1 cases

*Editor's Note: Shortly after publication of this article, the guidelines referred to were removed from the WHO Web site pending further peer review. They will be republished soon, according to the agency.*

Nov 29, 2006 (CIDRAP News) – The World Health Organization (WHO) this week released guidelines for investigating human cases of H5N1 avian influenza in an effort to shed more light on the mysterious virus and improve the chances of detecting changes that could turn it into a pandemic strain.

The 14-page document calls for a thorough probe of each case, from interviewing the patient and searching for contacts through hunting for other cases nearby and sifting data for any signs of human-to-human transmission. It calls for investigating suspected cases before laboratory test results are available.

The new guidance comes less than a month after the WHO issued a report on how much remains unknown about the H5N1 virus. Written by a group of leading flu experts, that report, issued Nov 2, said H5N1 illness is fundamentally different from ordinary flu in its severity and range of manifestations. Among other things, the panel called for research to determine why children and young adults seem especially susceptible and whether genetic factors increase the risk of infection or transmission among blood relatives.

The introduction to the new guidance says, "The document reflects and incorporates the practical field experience gained by investigators working at international, national, and sub-national levels during investigations of A(H5N1)."

The intention is that local health agencies will use the guidelines to build their own investigative plans and procedures, the WHO says.

The purposes of case investigation, according to the guidelines, include:

- Confirming the diagnosis of H5N1
- Reducing illness and death by rapidly identifying cases and starting appropriate treatment and precautions
- Reducing further spread by identifying possible sources of exposure and implementing prevention and control measures
- Determining if human-to-human transmission is becoming more efficient
- Identifying the key epidemiologic, clinical, and virologic characteristics of each case
- Ensuring timely exchange of information among clinicians, health investigators, and government officials to facilitate appropriate responses

In advice on searching for patients' contacts, the WHO says investigators should assume that patients are infectious for 1 week before onset of illness and 2 weeks afterward, even though the infectious period for the virus has not been determined. Healthy contacts should be monitored and given preventive antiviral treatment, depending on their risk of exposure, as defined in other WHO guidance.

Besides tracing contacts, investigators should search actively for other possible H5N1 cases by going from house to house and possibly conducting telephone surveys of healthcare facilities, practitioners, and labs, the WHO advises.

The agency recommends against using rapid diagnostic tests for the H5N1 virus. "The diagnostic accuracy of commercially available rapid tests is unknown, and if the test result is positive, differentiation between influenza A subtypes is not possible and confirmatory tests must be done," the document says.

In its Nov 2 report, the WHO said a rapid, reliable diagnostic test for H5N1 infection was urgently needed.

The guidance document discusses potential clues to a change in the virus's human transmissibility and what to look for in evaluating clusters of cases. It also suggests doing complementary studies, such as seroprevalence surveys of people with possible occupational risk in affected areas and case-control studies to evaluate risk factors for infection.

**See also:**

WHO guidelines for investigation of human cases of avian influenza A(H5N1)
http://www.who.int/csr/resources/publications/influenza/WHO_CDS_EPR_GIP_2006_4/en/index

Nov 2 CIDRAP News story "WHO report calls H5N1 vaccine stockpiling premature"

**Center for Infectious Disease Research & Policy**
Academic Health Center -- University of Minnesota
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# 医薬品 研究報告 調査報告書

| 識別番号・報告回数 | | | 報告日 | 第一報入手日 | 新医薬品等の区分 | 機構処理欄 |
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| | | | | 2007. 1. 22 | 該当なし | |

| 一般的名称 | (製造承認書に記載なし) | 研究報告の公表状況 | ProMED 20070120-0260, 2007 Jan 20. 情報源:[1]Kyodo News, 2007 Jan 18. | 公表国 |
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| 販売名(企業名) | 合成血「日赤」(日本赤十字社)<br>照射合成血「日赤」(日本赤十字社)<br>合成血-LR「日赤」(日本赤十字社)<br>照射合成血-LR「日赤」(日本赤十字社) | | | 日本 |

**研究報告の概要**

○日本(宮崎県)H5N1型高病原性トリインフルエンザ確定
2007年1月18日、農林水産省は、宮崎県の養鶏場で発生したトリインフルエンザは高病原性ウイルスによるものだったと明らかにした。同省は養鶏場で死亡した鶏から採取したウイルスのサンプルを検査して病原性が高いものであることを確認した。動物衛生研究所ではウイルスをH5N1型高病原性株と同定し、実験用鶏8羽にウイルスを接種したところ全て死亡したためこの結論に至った。H5N1型ウイルスの流行は、宮崎県清武町の谷口孵卵場黒坂農場で発生し、3つある鶏舎のうち1つで3500羽の鶏が死亡した。県の調査によると、他の養鶏場やペットの鳥などに異常は出ていない。

**使用上の注意記載状況・その他参考事項等**

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

| 報告企業の意見 | 今後の対応 |
|---|---|
| 宮崎県の養鶏場で発生したトリインフルエンザがH5N1型高病原性トリインフルエンザによるものだったと確定されたとの報告である。 | 日本赤十字社では家禽に高病原性トリインフルエンザの流行が認められた場合、当該飼養農場の関係者や防疫作業従事者の献血制限を行っている。新型インフルエンザが流行した場合、献血者減少につながることも予想される。今後も引き続き情報の収集に努める。 |

66

Back

**Archive Number** 20070120.0260
**Published Date** 20-JAN-2007
**Subject** PRO/AH/EDR> Avian influenza (13): Japan H5N1, Viet Nam, Indonesia

AVIAN INFLUENZA (13): JAPAN H5N1, VIET NAM, INDONESIA
**********
A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail, a program of the
International Society for Infectious Diseases
<http://www.isid.org>

[1] Japan (Miyazaki) - HPAI H5N1 confirmed
[2] Viet Nam, Can Tho
[3] Indonesia

******
[1] Japan (Miyazaki) - HPAI H5N1 confirmed
Date: Thu 18 Jan 2007
From: ProMED-mail <promed@promedmail.org>
Source: Kyodo News via Japan Times [edited]
<http://search.japantimes.co.jp/cgi-bin/nn20070119a4.html>

Bird flu virus in Miyazaki outbreak highly virulent
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The avian influenza that broke out last week at a poultry farm in Miyazaki Prefecture involved a highly virulent virus, the Agriculture, Forestry and Fisheries Ministry said on Thursday [18 Jan 2007]. The ministry determined the degree of virulence through a laboratory examination of virus samples taken from chickens that died of bird flu on the Miyazaki farm.

The National Institute of Animal Health, which identified the virus as the highly pathogenic H5N1 strain on Tuesday [16 Jan 2007], drew the conclusion after 8 chickens inoculated with the sampled virus were dead by Thursday [18 Jan 2007], the ministry said. Based in Tsukuba, Ibaraki Prefecture, the institute will continue genetic analysis for the consideration of the ministry's panel of experts.

The H5N1 strain, a subtype of the influenza A virus circulating basically in birds, has spread mainly in Asia and has killed more than 160 people in 10 countries since 2003, according to the World Health Organization. Its latest outbreak in poultry in Japan -- the 5th bird-flu case here since 2004 -- occurred at Taniguchi Furanjo Kurosaka Farm in Kiyotake, Miyazaki Prefecture, causing the death of 3500 birds in one of the farm's 3 poultry houses, mostly last week.

In the town of Kiyotake, inspectors from the Miyazaki Prefectural Government's task force Thursday [18 Jan 2007] checked bird-keeping households and homes within a 10 km radius of the farm, after they finished examining the 11 poultry farms with more than 1000 chickens in the area Wednesday [17 Jan 2007].

Ten households with at least 20 birds, including chickens and bantams [small breeds of chickens], were selected for Thursday's [18 Jan 2007] on-site inspections to take blood and fluid samples from 5 birds per household, task force officials said, adding that results will be released within a few days.

More than 150 households are breeding a total of around 1600 avian species in the town, including parakeets and other small birds kept as pets, town officials said.

******
[2] Viet Nam, Can Tho
Date: Fri 19 Jan 2007
From: Mary Marshall <tropical.forestry@btinternet.com>
Source: Reuters Alertnet [edited]
<http://www.alertnet.org/thenews/newsdesk/HAN277071.htm>

Bird flu found in ducks in Viet Nam city of Can Tho
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Bird flu has killed ducks in the southern Mekong delta's largest city of Can Tho, as Viet Nam's animal health experts increase measures to contain the H5N1 virus, a government report said on Friday [19 Jan 2007]. The Animal Health Department said that tests showed H5N1 had killed ducklings in Can Tho 4 days after the virus was found in neighboring Soc Trang province. The report said the domesticated ducks had not been vaccinated against the virus.

The Agriculture Ministry has ordered a new round of poultry vaccinations and sent more animal health experts to try to stem the bird flu, which has struck 7 provinces and Can Tho city in the southern rice basket region in the past month as a harvest nears.

This week, the government ordered farmers to stop ducks from roaming in the Mekong delta. Animal health officials said H5N1 has killed nearly 19 000 poultry in the delta, mostly ducks. Not all ducks are killed by the virus, some being infected without showing symptoms. The birds still excrete the virus in their droppings as they paddle through muddy rice paddies looking for insects and leftover grain.

Viet Nam has had no human H5N1 cases since November 2005, but the virus that 1st hit the south east Asian country in late 2003 returned to the Mekong delta last month [December 2006]. Thursday's [18 Jan 2007] Lao Dong (Labour) newspaper said 23 people who ate ducks that died from unknown causes in Bac Lieu province have been put under surveillance.

The government is anxious to stop bird flu spreading ahead of the Tet Lunar New Year festival in mid-February [2007], when poultry is part of the traditional feast. Officials fear the disease could spread nationwide on the wings of migrating birds and through the movement of poultry, including the smuggling of chickens and ducks from neighboring countries.

Bird flu killed 42 of the 93 people infected in Viet Nam in 2003-2005. It has killed 161 people out of 267 infections globally since 2003, World Health Organization figures show.

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[For Can Tho, see province 18 in the interactive map at <http://www.angelfire.com/co/hongnam/vnmap.html>. - Mod.AS]

******
[3] Indonesia
Date: Fri 19 Jan 2007
From: Mary Marshall <tropical.forestry@btinternet.com>
Source: Reuters Alertnet [edited]
<http://www.alertnet.org/thenews/newsdesk/JAK212987.htm>

Indonesian capital's bird cull gets mixed reaction
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A campaign to rid the Indonesian capital of backyard fowl to fight bird flu got a mixed reaction from residents on Friday [19 Jan 2007], with some welcoming it, while others worried about losing a key source of income.

Jakarta governor Sutiyoso set fire to about 1600 chickens, ducks, and pigeons that had been slaughtered and dumped into a pit overnight in a densely populated neighborhood. Some residents responded with cheers of "Hurray!" at the launch of the campaign, although the culled birds were not known to have been infected by bird flu.

Sutiyoso told city residents on Wednesday [17 Jan 2007] that their backyard

fowl would be confiscated and destroyed if they failed to get rid of the birds by the end of the month. The move followed the deaths of 4 people in Jakarta and its surrounding areas since the start of the year [2007], which took the number of confirmed human deaths from the virus in Indonesia to 61, the highest in the world. "We support the government's program. It's better to do this than worrying about being infected," a resident said. But some residents were worried about the cost. Many people in Indonesia keep poultry to supplement their meager incomes. "The program is good but how can we eat eggs but not chicken?" she asked.

A trader at a nearby bird market said he had not sold a single bird since the governor announced the ban. He said that, even though traders like him could still sell birds, traders and owners would need to get a clean bill of health for birds from authorities. This could discourage buyers. "I hope officials can be present here to facilitate the certification," he said.

Health minister Siti Fadilah Supari on Thursday [18 Jan 2007] said the ban in Jakarta would be extended to 8 other provinces that had reported human infections of the H5N1 bird flu virus. More than a dozen people have been admitted to hospital with bird flu-like symptoms since the start of the year [2007], although many have been discharged or tested negative for the virus.

Indonesia faces an uphill task controlling the disease. Millions of backyard fowl live in close proximity to humans, and health education campaigns have often been patchy and rules difficult to enforce. Illustrating this, chickens roam freely just a few minutes walk from the capital's central business district.

Official calls for culling have also met stiff resistance in the past due to meager compensation and difficulties enforcing rules in the provinces. Officials said poultry owners would be paid compensation of 12 500 rupiah (USD 1.4) for each sick bird killed. A fully grown chicken costs about 35 000 rupiah [USD 4] in Jakarta. The governor said on Wednesday [17 Jan 2007] that there would be no payment for healthy birds.

[byline: By Heru Asprihanto]

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[No cases of avian influenza have been detected in Europe during the current winter season. It will be interesting to follow the results of this year's (2007) EU surveillance in wild birds. In 2006, infected wild birds were detected between February and May. Avian influenza is one of the main subjects for discussion during the due 25th Plenary meeting of EFSA'a (European Food Safety Authority) Animal Health & Animal Welfare panel, to be convened in Parma, Italy, 31 Jan - 1 Feb 2007. Among the topics: avian influenza - zoo birds vaccination; avian influenza vaccines; situation - migratory birds. - Mod.AS]

[see also:
Avian influenza (12): Hong Kong, Thailand, Japan, OIE 20070118.0237
Avian influenza, human (14): Indonesia, WHO 20070116.0204
Avian influenza (06): Viet Nam, OIE 20070113.0175
Avian influenza (02): Viet Nam 20070105.0050
Avian influenza: Viet Nam (Bac Lieu, Ca Mau, Hau Giang) 20070102.0015
Avian influenza (08): Japan (Miyazaki), H5 20070114.0184
2006
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Avian influenza (225): Viet Nam (Bac Lieu, Ca Mau, Hau Giang) 20061231.3662]

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| 一般的名称 | ①乾燥抗HBs人免疫グロブリン<br>②ポリエチレングリコール処理抗HBs人免疫グロブリン | | 研究報告の公表状況 | Transfusion 2007; 47(3): 452-459 | 公表国<br>オーストリア | |
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**研究報告の概要**

ヒトのH5N1ウイルス感染の増加が継続していることによって、これらウイルスがヒトの間で効率的に伝播する能力を獲得し、流行を起こす可能性がある。最近、より組織的なウイルスの伝播が、ヒトのH5N1ウイルス感染の間で報告され、その血液中に顕著なウイルス濃度が観察された。このことによって、輸血用血液製剤、ひいては血漿分画製剤の安全性についての懸念が高まった。
血漿分画製剤の安全性のマージンを確認するため、製造中のウイルス不活化工程について、ウイルスの不活化効果をH5N1インフルエンザウイルスの再集合体株を使って調査した。
その結果は、H5N1インフルエンザウイルスは、ヒトアルブミンの液状加熱、静注グロブリンのSD処理及び低pHインキュベーション、並びに第VIII因子インヒビターバイパス複合体製剤（FEIBA）の蒸気加熱工程において、HIV、BVDV、及びPRVのエンベロープウイルスと同様の挙動を示し、効率的に不活化された。
すなわち、H5N1インフルエンザウイルスの理論的伝播に対する血漿分画製剤の安全性マージンは、十分なものであった。

**使用上の注意記載状況・その他参考事項等**

代表として静注用ヘブスブリンーIHの記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| ウイルス不活化実験の結果、H5N1インフルエンザウイルスは、HIV、BVDV、及びPRVのエンベロープウイルスと同様の挙動を示したとの報告である。<br>血漿分画製剤からの高病原性トリインフルエンザA（H5N1）ウイルス伝播の事例は報告されていない。また、万一原料血漿に高病原性トリインフルエンザウイルス(H5N1)が混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

67

# H5N1 influenza virus and the safety of plasma products

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**BACKGROUND:** The ever-increasing number of human H5N1 influenza virus infections may enable these viruses to acquire the ability to spread effectively among humans and potentially to cause a pandemic. Recently, more systemic virus dissemination was reported during H5N1 virus infection of humans, resulting in significant virus concentrations also in the blood. The observation has raised concerns about the safety of labile blood products for transfusion and consequentially also for plasma derivatives. To confirm the safety margins of plasma products, dedicated virus inactivation processes used during their production were investigated for their effectiveness in inactivating this virus of recent concern.
**STUDY DESIGN AND METHODS:** Virus inactivation by steps commonly used during the manufacture of plasma derivatives, such as pasteurization for human albumin, solvent/detergent treatment for intravenous immunoglobulin (IVIG), vapor heating for factor VIII inhibitor bypassing activity, and incubation at low pH for IVIG, were investigated with a reassortant strain of H5N1 influenza virus.
**RESULTS:** The results show that H5N1 influenza behaves as expected for lipid-enveloped viruses; that is, the virus is effectively inactivated by all the commonly used virus inactivation procedures tested.
**CONCLUSION:** The safety margins of plasma derivatives against the theoretical transmission of H5N1 influenza virus are very substantial.

Influenza A viruses circulate in wild birds around the world and mostly coexist with their hosts. Since 1996, however, H5N1 viruses were recognized that had evolved into being more pathogenic for poultry and to occasionally also affect humans. To date, approximately 140 million domesticated birds have died from H5N1 virus infection or culling to prevent further virus spread,[1] and according to the WHO, of 233 humans reported infected 135 have died as of August 7, 2006.[2] This rather unusual pathogenicity of these H5N1 viruses for humans has been attributed to a resistance to the antiviral effects of interferons and tumor necrosis factor.[3] Also, H5N1 viruses that infect humans seem to have a broader tissue tropism when tested in ferrets[4] but also in the humans affected.[5,6] Once newly emerging influenza viruses acquire the ability to effectively spread among humans, these viruses can cause pandemics associated with significant morbidity and mortality as testified by several such episodes during the past century. Beyond those directly affected by virus infection, however, influenza viruses have generally been considered a relatively minor concern with respect to the safety of the blood supply, for example, based on the conclusions that viremia appears to be uncommon and that if viremia does occur it will be associated with fever and severe illness so that it is unlikely that these folks are going to want to donate.[7]

Careful review of available literature on the topic though, including less current and thus more difficult to access information, reveals several instances of reported influenza virus viremia in humans,[8-11] including cases where viremia has been described to occur in the absence of medical symptoms.[8,11] Reported several decades ago this information is still less comforting, although given our at present limited experience with H5N1 influenza, the relevance of the information to the current situation is not clear.

Recently, however, the presence—though not replication—of H5N1 virus was demonstrated in the spleen of an ultimately fatal human case, a finding that would support hematogenous spread of the virus.[5] Along those lines, the occurrence of influenza virus in the blood has been reported in two fatal cases of human H5N1 infection, at approximately 85,000[6] and 3,080[12] polymerase chain reaction–detectable copies per mL.

**ABBREVIATIONS:** BVDV = bovine viral diarrhea virus; FEIBA = factor VIII inhibitor bypassing activity; HA = human albumin; PRV = pseudorabies virus.

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Based on currently available scientific evidence it would appear that one cannot be too certain about the level of concern that the H5N1 influenza viruses will, or not, pose to the safety of the blood supply, and by inference also the safety of plasma-derived medicines. For plasma-derived medicines though, a product class under particular scrutiny as it has in the past been involved in amplifying the spreading of emerging agents, additional reassurance can be derived from the dedicated inactivation procedures that are required to be incorporated into their manufacturing processes.[13] Although these processes have been validated for their effectiveness in inactivating other lipid-enveloped viruses, the available experimental evidence with orthomyxoviruses is very limited and to the author's best knowledge none for influenza H5N1 viruses. Validation studies with a range of physicochemically diverse viruses,[14] so-called model viruses, however, have generated evidence that these steps would provide very significant margins of safety also against H5N1 viruses for these products.

To provide additional reassurance, however, particularly for the patients who critically depend on plasma-derived products, but also regulatory authorities who need to justify the safety and thus presence of plasma derivatives on the market, studies aimed at confirming that influenza H5N1 viruses themselves would indeed behave as expected were performed for several major virus inactivation steps currently used during the manufacture of plasma derivatives.

## MATERIALS AND METHODS

### Viruses, cells, and infectivity assays

The human immunodeficiency virus (HIV), strain IIIB (National Institutes of Health [NIH], Bethesda, MD), was propagated on H9 cells (European Collection of Cell Cultures, Salisbury, UK; 85050301) and titrated on the Epstein-Barr virus transformed B lymphoblastoid cell line AA-2 (AIDS Research and Reference Reagent Program, NIAID, NIH, Bethesda, MD).

The bovine viral diarrhea virus (BVDV), strain Kentucky 22 (Biological Research Faculty & Facility, Ijamsville, MD), was propagated on MDBK cells (American Type Culture Collection [ATCC], Manassas, VA; CCL-22) and titrated on BT cells (ATCC CRL-1390). The pseudorabies virus (PRV), strain Kaplan (Federal Research Center for Virus Diseases of Animals, Tübingen, Germany), was propagated and titrated on Vero cells (European Collection of Cell Cultures 84113001).

The H5N1 influenza virus, strain NIBRG-14 (provided by J.M. Wood and J.S. Robertson from the National Institute for Biological Standards and Control, Potters Bar, Hertfordshire, UK), was propagated on embryonated eggs and titrated on MDCK cells (ATCC CCL-34). NIBRG-14 H5N1 virus has been derived as a vaccine reference strain by reverse genetics, that is, the transfection of different influenza virus genes into one host cell that then produces the newly assembled virus (essentially as described in Nicolson et al.[15]). With respect to pathogenicity, the NIBRG-14 strain is very different from the currently circulating highly pathogenic H5N1 viruses, in that it is apathogenic. The virus particles, however, are physicochemically equivalent between these two viruses, as the surface glycoproteins hemagglutinin and neuraminidase are of H5N1 origin.

Virus-containing samples were titrated by $TCID_{50}$ assays with serial half-log dilutions of samples. Cells were incubated for 6 ± 1 days with samples before the cytopathic (BVDV, PRV, H5N1) or fusogenic (HIV) effect induced by the respective virus was evaluated. Virus concentrations were calculated according to the Poisson distribution and expressed as log ($TCID_{50}$) per mL.

### Downscaled manufacturing processes for plasma derivatives

Downscaled versions of the manufacturing processes investigated were established, and their equivalence to the respective large scale processes was demonstrated by comparing critical process parameters and selected product parameters. Temperature as a critical process parameter for virus inactivation was monitored throughout all the processes investigated. The concentrations of virus-inactivating chemicals and the pH were measured, where relevant, to confirm equivalence with the manufacturing scale processes. Process intermediates were obtained from the manufacturing scale and used as starting materials, which were spiked 1:10 with virus stock suspensions. Immediately after spiking, samples were drawn and titrated to confirm the amount of virus added. Further samples were collected and titrated immediately at certain points during and at the end of the virus inactivation processes. Corresponding unspiked process intermediates were obtained from control runs and tested for their potential cytotoxicity for indicator cell lines and for their potential interference with the detection of low virus titers. Virus reduction factors for the manufacturing processes examined were calculated in accordance with Committee for Proprietary Medicinal Products (CPMP) guidance.[14]

### Pasteurization of human albumin

During the pasteurization of final container of human albumin (HA), the liquid product is heat treated for 600 to 700 minutes at 60°C. Virus inactivation was investigated by heating 50 to 60 mL of the final product at 58°C, that is, 2°C below the temperature specified for manufacturing, for 600 minutes, which is the shortest possible treatment time. To provide further confidence in the robustness of the virus inactivation achieved, the heating process was evaluated with product of low and the highest HA concentrations, that is, 3.5 or 5 percent and 25 percent.

### Vapor heating of anti-inhibitor coagulant complex, that is, factor VIII inhibitor bypassing activity

During the manufacture of factor (F)VIII inhibitor bypassing activity (FEIBA), a lyophilized intermediate of low residual moisture is heat treated in two phases, that is, first for a minimum of 510 minutes at 60°C, followed secondly by 60 minutes at 80°C. For the downscaled process, these heating steps were performed at the lower limit of these temperature specifications and for incubation times just below those specified for the manufacturing scale process. Also, separate runs were performed at the upper and lower limit of the residual moisture content as specified for manufacture to provide further assurance relative to the virus inactivation potential of the process. Product intermediate was spiked with virus, lyophilized, and then heat treated according to the procedure described above. FEIBA (clotting assay), FII activity (clotting assay), and FX activity (chromogenic assay), and protein concentration were determined for the downscale intermediate before and after the vapor-heating process, and the results were compared to the respective values for intermediates from the manufacturing scale to confirm equivalence of the different scale processes.

### Solvent/detergent treatment of intravenous immunoglobulin (IVIG), that is, Gammagard Liquid/KIOVIG

During the intravenous immunoglobulin (IVIG), that is, Gammagard Liquid (Baxter Healthcare Corp., Westlake Village, CA)/KIOVIG (Baxter AG, Vienna, Austria), manufacturing process, intermediate product is incubated with the solvent/detergent (S/D) chemicals tri-*n*-butyl phosphate, octoxynol-9, and polysorbate 80, at target concentrations of 0.3, 1, and 0.3 percent, respectively, for at least 60 minutes at 18 to 25°C and pH 5.2. As earlier studies had shown virtually immediate virus inactivation under these conditions, only 50 and 5 percent of the nominal concentration of the virus-inactivating chemicals were used in experimental runs to provide a meaningful assessment of the kinetics of model and target virus inactivation. The equivalence of the downscale and manufacturing scale processes was further supported by monitoring of parameters like conductivity, pH, temperature, and measurement of the protein concentrations of the respective intermediates.

### Low-pH treatment of IVIG (Gammagard Liquid/KIOVIG)

In the course of the manufacture of this IVIG preparation, the final product is incubated at a low pH of 4.4 to 4.9 and a temperature of 30 to 32°C for 21 to 22 days. To investigate virus inactivation by this procedure, the manufacturing scale intermediate was incubated at 29°C, that is, at the lower limit of the process specification, and at pH values of 4.4 and 4.9, that is, at the lower and the upper limit defined for the manufacturing scale process, and for up to 21 days, that is, the minimal incubation time for manufacture. To verify the equivalence of the downscale with the manufacturing scale process, pH and temperature, parameters critical for virus inactivation, were monitored throughout the incubation time.

## RESULTS

### Pasteurization of HA

Per European and US Pharmacopoeia requirements, the final HA product is subjected to pasteurization as a dedicated virus inactivation step. To create conditions least favorable to virus inactivation during the experimental investigations, the product was heat treated at only 58°C instead of at the 60°C process temperature specification.

In duplicate runs of 600 minutes' duration, with either low- (3.5 or 5%) or high-concentration (25%) HA intermediates, all the HIV, BVDV, and PRV that could be spiked into the product at the lower/upper limit of the specified protein concentration were completely inactivated after 30/30, 120/120, and 30/30 minutes, respectively, of the 600-minute heating process. Also, the H5N1 influenza virus was completely inactivated after 30/30 minutes of heating within material (Table 1). The reduction factors after the full 600-minute heating period were greater than 8.0/greater than 7.5 for HIV, greater than 6.4/greater than 6.2 for BVDV, greater than 7.7/greater than 7.2 for PRV, and greater than 7.6/greater than 7.0 for H5N1 influenza virus.

### Vapor heating of FEIBA

Intermediate FEIBA was subjected to vapor heating in a downscaled process at temperatures and incubation times just below the specifications for these parameters during the manufacturing scale processes, in runs performed at residual moisture levels of 7 and 8 percent, that is, the lower and upper limit for manufacturing. Nevertheless, the combination of lyophilization and subsequent vapor heating inactivated all the model viruses as well as the H5N1 influenza virus to below the limit of detection during the two-phase heat treatment (Table 2), resulting in virus reduction factors of greater than 5.9/greater than 5.8 for HIV, greater than 5.6/greater than 5.6 for BVDV, greater than 6.7/greater than 6.6 for PRV, and greater than 5.3/greater than 5.1 for H5N1. Virus inactivation by the two-phase vapor-heating process was reconfirmed as being insensitive to slight variations in residual moisture content of the intermediates, in that there was no significant difference between virus inactivation at the upper

**TABLE 1. Virus inactivation during pasteurization of HA***

| | HIV | | BVDV | | PRV | | H5N1 | |
|---|---|---|---|---|---|---|---|---|
| HA concentration (%): | 5 | 25 | 5 | 25 | 5 | 25 | 3.5 | 25 |
| Virus stock suspension | 8.0 | 8.0 | 7.1 | 7.1 | 7.4 | 7.4 | 7.3 | 7.3 |
| Spiked process intermediate | 6.9 | 7.0 | 6.0 | 5.8 | 6.7 | 6.6 | 6.6 | 6.5 |
| Heating at | | | | | | | | |
| 58°C for 0-1 min | 5.4 | 4.8 | 4.5 | 4.2 | 1.6 | 0.9 | 2.9 | 3.0 |
| 58°C for 30 min | <0.1 | <0.6 | 1.8 | 0.6 | <0.1 | <0.6 | <0.1 | <0.6 |
| 58°C for 60 min | <0.1 | <0.6 | 0.6 | 0.6 | <0.1 | <0.6 | <0.1 | <0.6 |
| 58°C for 120 min | <0.1 | <0.6 | <0.6 | <0.6 | <0.1 | <0.6 | <0.1 | <0.6 |
| 58°C for 360 min | <0.1 | <0.6 | <0.6 | <0.6 | <0.1 | <0.6 | <0.1 | <0.6 |
| 58°C for 600 min | <0.1 | <0.6 | <0.6 | <0.6 | <0.1 | <0.6 | <0.1 | <0.6 |
| 600 min (bulk titration) | <(–0.8) | <(–0.3) | <(–0.3) | <(–0.3) | <(–0.8) | <(–0.3) | <(–0.8) | <(–0.3) |
| Reduction factor (log)† | >8.0 | >7.5 | >6.4 | >6.2 | >7.7 | >7.2 | >7.6 | >7.0 |

* Results are reported as $TCID_{50}$ per mL. Negative log values for virus titers can be obtained when volumes greater than 1 mL are used for virus titration, for example, 1 infectious unit in 10 mL equals 0.1 unit per mL; the log of 0.1 is (–1). The same is true for the detection limit when no infectious virus is detected.

† Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

**TABLE 2. Virus inactivation during freeze-drying followed by vapor heating of FEIBA***

| | HIV | | BVDV | | PRV | | H5N1 | |
|---|---|---|---|---|---|---|---|---|
| Residual moisture (%): | 7.0 | 8.0 | 7.0 | 8.0 | 7.0 | 8.0 | 7.0 | 8.0 |
| Virus stock suspension | 7.4 | 7.2 | 7.0 | 6.7 | 8.0 | 7.7 | 6.3 | 6.5 |
| Spiked process intermediate | 6.2 | 6.1 | 6.0 | 5.5 | 7.1 | 7.0 | 5.3 | 5.0 |
| Spiked and lyophilized intermediate | 4.6 | 4.8 | 3.2 | 3.5 | 4.8 | 4.6 | 4.4 | 4.4 |
| Heated to 59.0°C | 3.5 | 3.2 | 2.7 | 2.6 | 1.1 | 1.4 | 4.2 | 4.2 |
| Heated at | | | | | | | | |
| 59.5 ± 0.5°C for 180 min | 1.9 | 2.0 | 1.1 | 0.9 | 1.8 | <1.1 | 2.9 | 2.7 |
| 59.5 ± 0.5°C for 360 min | 1.7 | 1.8 | <1.1 | <0.6 | <1.1 | <1.1 | 2.0 | <0.6 |
| 59.5 ± 0.5°C for 505 min | 1.9 | 1.4 | <1.1 | <0.6 | <1.1 | <1.1 | <0.6 | <0.6 |
| Heated to 79.0°C | 0.6 | 0.6 | <1.1 | <0.6 | <1.1 | <1.1 | <0.6 | <0.6 |
| Heated at | | | | | | | | |
| 79.5 ± 0.5°C 30 min | <0.6 | <0.6 | <1.1 | <0.6 | <1.1 | <1.1 | <0.6 | <0.6 |
| 79.5 ± 0.5°C 55 min | <0.6 | <0.6 | <1.1 | <0.6 | <1.1 | <1.1 | <0.6 | <0.6 |
| Reduction factor (log)† | >5.9 | >5.8 | >5.6 | >5.6 | >6.7 | >6.6 | >5.3 | >5.1 |

* Results are reported as $TCID_{50}$ per mL.

† Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

and the lower limits for the moisture content specified for the manufacturing procedure.

### S/D treatment of Gammagard Liquid/KIOVIG

Investigating the S/D treatment of IVIG intermediates S/D chemicals were used at significantly reduced concentrations compared to those specified for the manufacturing scale process. By use of only half of the nominal concentrations of the S/D chemicals, that is, the minimum concentration allowable for manufacturing, BVDV, PRV, and H5N1 influenza were inactivated to below the limit of detection already after 2 minutes of the 60-minute treatment and HIV after 10 minutes. In an even more drastic experimental setting, only 5 percent of the S/D chemicals were added. Even these 20-fold lower concentrations compared to manufacturing resulted in a complete inactivation of HIV, BVDV, and H5N1 influenza, for BVDV and H5N1 already after 2 minutes of the incubation period. PRV was inactivated by 2.8 log. Because partial H5N1 inactivation occurred already after spiking the virus into the test article at pH 5.2, that is, before addition of the S/D chemicals, an additional experiment was performed with process intermediate rebuffered to neutral pH (7.0-7.4). As can be seen, the presence of 5 percent of the S/D chemicals compared to manufacturing still inactivated 1.9 log H5N1 influenza virus (Table 3).

### Low-pH treatment of Gammagard Liquid/KIOVIG

When investigating the low pH treatment of the IVIG intermediate, HIV, BVDV, and PRV were completely inactivated by the low-pH treatment procedure, at pH values at both the upper and the lower limit of the manufacturing process specifications. The incubation times and temperatures applied in the downscale investigation were again those least favorable for virus inactivation, that is, the lowest possible temperatures and shortest possible incubation times (Table 4). The H5N1 virus, however, was inactivated instantaneously, as expected from the known

**TABLE 3. Virus inactivation during S/D treatment of Gammagard Liquid/KIOVIG***

| | HIV | | BVDV | | PRV | | H5N1 | | |
|---|---|---|---|---|---|---|---|---|---|
| SD chemicals (% of specified): | 50 | 5 | 50 | 5 | 50 | 5 | 5 | 50 | 5 |
| pH value | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 5.2 | 7.0-7.4† |
| Virus stock suspension | 7.9‡ | 7.9 | 8.3‡ | 8.1 | 8.0‡ | 7.7 | 7.2 | 7.4 | 7.3 |
| Spiked process intermediate | 7.6‡ | 7.6 | 8.6‡ | 8.2 | 7.8‡ | 8.4 | 6.3‖ | 3.1‖ | 7.1 |
| 2 min§ | 4.5‡ | 5.0 | <3.7‡ | <3.7 | <4.0‡ | 7.0 | <5.7 | <3.7 | 6.2 |
| 10 min§ | <4.5‡ | 4.9 | <3.7‡ | <3.7 | <4.0‡ | 6.4 | <5.2 | <3.7 | 5.7 |
| 20 min§ | <4.5‡ | 4.5 | <3.7‡ | <3.7 | <4.0‡ | 6.0 | <5.2 | <3.7 | 5.7 |
| 30 min§ | <4.5‡ | 3.7 | <3.7‡ | <3.7 | <4.0‡ | 6.1 | <4.7 | <3.7 | 5.9 |
| 60 min§ | <4.5‡ | <3.7 | <3.7‡ | <3.7 | <4.0‡ | 5.7 | <4.7 | <3.7 | 5.2 |
| 30 min (bulk titration)§ | <3.6‡ | ND | <2.8‡ | <2.8 | <3.1‡ | ND | <3.8 | <2.8 | ND |
| 60 min (bulk titration)§ | <3.6‡ | ND | <2.8‡ | <2.8 | <3.1‡ | ND | <3.8 | <2.8 | ND |
| Reduction factor (log) | >4.5‡¶ | >3.9 | >6.2¶ | >5.8¶ | >5.1‡¶ | 2.8 | >3.7¶ | >4.7¶ | 1.9 |

* Results are given as $TCID_{50}$. ND = not determined
† Because H5N1 is inactivated at pH 5.2, an additional virus-spiked run was performed with intermediate rebuffered to pH 7.0 to 7.4.
‡ Mean of two runs performed with intermediate at the low or high end of the protein concentration specification.
§ Sample pretreatment, that is, samples containing S/D reagent were diluted 1:100 with 2 to 8°C cold cell culture medium prior to titration, was taken into account.
‖ The titer of the "spiked process intermediate" sample from the pH 7.0 to 7.4 run was used for calculation of the reduction factor.
¶ Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).

**TABLE 4. Virus inactivation during low-pH incubation of Gammagard Liquid/KIOVIG***

| | HIV | | BVDV | | PRV | | H5N1 | |
|---|---|---|---|---|---|---|---|---|
| pH: | 4.4 | 4.9 | 4.4 | 4.9 | 4.4 | 4.9 | 4.4 | 4.9 |
| Virus stock suspension | 6.7 | 6.7† | 6.8 | 6.7† | 7.4 | 7.4† | 6.9 | 6.9 |
| Spiked process intermediate | 5.5 | 5.5† | 5.7 | 5.8† | 6.4 | 6.3† | <1.1 | <1.1 |
| 0 days (after reaching temperature)‡ | 4.9 | 5.4† | 5.6 | 5.6† | 5.5 | 6.2† | <1.6 | <1.6 |
| 1 day‡ | ND | ND | ND | ND | 0.6 | 1.0† | <0.6 | <0.6 |
| 2 days‡ | <0.6 | <0.9† | ND | ND | <1.1 | <0.9† | ND | ND |
| 4 days‡ | <1.1 | <0.9† | 3.4 | 4.4† | <1.1 | <0.9† | <0.6 | <0.6 |
| 7 days‡ | <1.1 | <0.9† | 3.1 | 2.9† | <1.1 | <0.9† | <0.6 | <0.6 |
| 10 days‡ | <1.1 | <0.9† | ND | ND | <1.1 | <1.1† | <0.6 | <0.6 |
| 14 days‡ | <1.6 | <1.1† | <1.6 | 1.1† | <1.6 | <1.1† | <1.1 | <1.1 |
| 20 days‡ | <1.1 | <1.1† | <1.6 | <1.1† | <1.6 | <1.4† | <1.1 | <1.1 |
| 14 days (bulk titration)‡ | <0.7 | <0.2† | <0.7 | NA/<0.7†§ | <0.7 | <0.2† | <0.2 | <0.2 |
| 20 days (bulk titration)‡ | <0.2 | <0.2† | <0.7 | <0.2† | <0.7 | <0.5† | <0.2 | <0.2 |
| Reduction factor (log)‖ | >5.7 | >5.8† | >5.4 | >5.8† | >6.4 | >6.6† | >6.3¶ | >6.3¶ |

* Results are given as $TCID_{50}$ per mL. ND = not determined; NA = not applicable.
† Mean of two runs.
‡ Sample pretreatment, that is, 1:3.16 dilution with 2 to 8°C cold cell culture medium before titration, was taken into account.
§ For one of the two runs, no titer could be calculated for the bulk titration due to the presence of positive wells.
‖ Calculation of reduction factors was done including titers of cumulative negative samples (details not shown).
¶ Titer of "virus stock suspension" was used for calculation of the reduction factor, because complete H5N1 virus inactivation occurred already after spiking.

pH-dependent activation of the influenza virus hemagglutinin at pH 5.2 or lower.[16]

## DISCUSSION

During the licensing process the safety margins of plasma-derived medicinal products with respect to potential virus transmissions are validated by generating and/or assessing experimental information on the behavior of a range of physicochemically diverse viruses, that is, model and target viruses, in downscaled models of the respective manufacturing processes. When facing emerging viruses, this information, which has inherently been designed to address different concerns, still forms the basis of predictions relative to the behavior of these other less well-known viruses. And while there is a notion that the plentiful information generated with a wide variety of viruses does still, at least for the more widely used inactivation procedures, allow for reasonably solid extrapolations towards which viruses should be more susceptible or resistant to these procedures, any newly emerging virus challenges these assumptions in a rather discomforting way.

With respect to influenza viruses, orthomyxoviruses have not been widely used as model viruses, and thus specifically for these viruses the available amount of information is limited, at best. For H5N1 itself, the available amount of information has been none. The NIBRG-14 virus used for the current investigation is physicochemi-

cally equivalent to currently circulating highly pathogenic H5N1 viruses, as defined by the surface proteins H5 and N1, as well as an equally host cell–derived virus envelope. With respect to a minor structural difference, that is, the absence of the polybasic cleavage site from the NIBRG-14 hemagglutinin, others have shown that influenza A virus strains that contain such noncleaved hemagglutinins can even be somewhat more resistant to inactivation by, for example, low pH,[17] which would make predictions of product safety versus wild-type viruses based on the data of the current study even more robust. Most importantly though, where the presented experiments have determined virus inactivation on a logarithmic scale, any minor differences between the virus strains will be inconsequential for the general conclusions reached. In contrast to these structural and thus physicochemical similarities, the pathogenicity of the NIBRG-14 virus is significantly different to the currently circulating highly pathogenic H5N1 viruses (see Materials and Methods); that is, it is none. This combination of properties has greatly facilitated performing the laboratory work described, in that for the first time inactivation data could be generated with an H5N1 influenza virus strain without safety concerns for the operators/the environment.

Even before the availability of H5N1 inactivation data, however, several other aspects have argued against the relevance of H5N1 or more generally influenza viruses for the safety of plasma products. First, the occurrence of influenza virus in blood, that is, viremia, has been considered "... uncommon ...,"[7] although several less recent reports have described the very fact[8-11] and even viremia during the preclinical course of influenza virus infection,[8,11] which would represent a setting of utmost relevance for the safety of all blood-derived medicinal products. Importantly though, all this information has been derived from influenza viruses different from the bird influenza virus H5N1.

Second, the virus titers reported during viremia were generally rather lower than those of the viruses established as relevant for transfusion safety, although the notion was challenged by the recently reported approximately 85,000 copies per mL as determined for a serum specimen from a fatal human H5N1 case.[6] Another similar case, however, found a virus titer in plasma of only approximately 3,080 copies per mL.[12] It is emphasized that both of these specimens were obtained from terminally ill patients, that is, under circumstances not applicable for the collection of blood or plasma. Determining the potential viremia levels in asymptomatic individuals remains a research priority, yet expectedly those titers will be lower than those found in individuals in late or even terminal stages of disease.

Third, extensive studies of other lipid-enveloped viruses have indicated that these viruses are easily inactivated and should thus not be considered a concern to the safety of plasma derivatives. The concerns of particularly patient groups whose treatment depends on the safety of plasma-derived products, but also regulators whose remit includes to guarantee the safety of these medications, are nevertheless understandable in view of the history of plasma products.

Based on the data presented the safety margins of plasma products in the event of an H5N1 influenza pandemic can be estimated, following the basic principles set forth in regulatory guidance.[18] With respect to "potential virus input"[18] into the manufacturing process, neither the prevalence of viremic potential blood or plasma donors nor the potential virus load of a donation obtained under such circumstances is known at this time. The level of influenza viremia during preclinical stages of infection, however, can safely be assumed to be lower than those detected in terminally ill individuals, that is, approximately 85,000[6] and 3,080[12] copies per mL, and generally influenza viremia would be less likely in preclinically infected humans who would have qualified as donors. More specific information about the prevalence of viremia in blood or plasma donors as well as peak levels of viremia is necessary to develop more precise assessments and will hopefully become available quickly once a potential influenza pandemic commences.

Regarding the "overall virus inactivation/removal capacity"[18] all the H5N1 virus that could be spiked into experimental downscales to evaluate standard virus inactivation technologies was completely inactivated well before the end of these processes, with virus reduction factors in the range of greater than 4.7 to greater than 7.6 log, that is, well beyond the widely held effectiveness threshold "... of the order of 4 logs or more. . ."[14] Regulations suggest that "... it will be desirable in many cases to incorporate two distinct effective steps ..." into any manufacturing process,[13] which would result in a cumulative reduction of potential virus load by greater than 8 log, that is, by more than 100 million-fold. Given these numbers the safety margins that these dedicated virus reduction techniques would ensure for plasma derivatives become intuitively clear.

Given the central importance of virus reduction for the safety of plasma derivatives, the current investigation thus sought to experimentally confirm the expected behavior for H5N1 influenza virus, in examining the inactivation potential of procedures widely used throughout the plasma products industry against the H5N1 influenza virus itself. Reassuringly, all the inactivation methods tested were shown to completely inactivate all the virus that could be spiked into the respective product intermediates.

Pasteurization was found to be extremely effective in inactivating all the lipid-enveloped viruses tested, that is, HIV, BVDV, PRV, and also H5N1, at both the upper and the lower limits of the specified protein concentration (Table 1).

Vapor heating, a proprietary heat treatment that is used, for example, during the FEIBA manufacturing procedure, was also confirmed to be extremely effective. The viruses tested were completely inactivated before initiation of (BVDV, PRV, H5N1) or early during (HIV) the second heat treatment phase, which thus provides extra margins of safety. Of note, the results obtained would indicate that H5N1 is more stable during lyophilization than is BVDV or PRV (Table 2).

The results obtained for the S/D treatment of IVIG agreed with earlier findings that have established this method as most effective in inactivating lipid enveloped viruses (Table 3). Also, the rather lower pH of the plasma products intermediate tested, that is, pH 5.2, which is equivalent to the intermediate during large scale manufacturing, was realized to contribute to the inactivation of the H5N1 influenza virus (Table 3).

For the low-pH treatment investigated (Table 4), the H5N1 was inactivated particularly effectively. This is not surprising because during the infection cycle of influenza viruses a low pH–induced fusion event is needed for the virus to escape from the late endosome into the cytosol, and if this event occurs outside the susceptible cell, the virus would be rendered noninfectious.[16]

Although not directly investigated in the experiments presented, influenza viruses would, based on their approximate size of 80 to 120 nm,[19] also be expected to be removed by the now widely used nanofilters (virus filters) with nominal pore sizes in the 15- to 75-nm range and typically between 35 and 50 nm.

Together, the data presented should alleviate any H5N1-associated concerns about the safety of plasma derivatives and will hopefully support an evidence-based decision making process in all further dealings with the emergence of influenza viruses. The results will, however, have to stand their test when a possible first blood transfusion transmission of an influenza virus would be documented. Beyond this discussion of the safety of blood and plasma-derived products, the impact of a potential influenza pandemic on the supply of these life-sustaining products needs to be kept in mind. A recently presented model calculation from the US Centers for Disease Control and Prevention (CDC) estimated a donor shortfall of, depending on the specific scenario envisaged, as much as 15 to 20 percent for up to 8 weeks (M. Meltzer, CDC; presented at the PPTA Plasma Forum, June 20, 2006, Washington DC). It is critical, therefore, that all current pandemic influenza preparedness efforts include the provision of adequate supplies of blood and plasma derivatives into their goals.

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**研究報告の概要**

<背景>
中国は、20世紀前半に梅毒の大きな流行を経た後、1960～80年の20年間、この感染を排除することができた。しかし、中国社会の大きな変化の後、性感染症の流行が再発した。散発的に発行された報告によって梅毒流行の重大性についての警鐘が鳴らされてきたが、計画及び介入のためのデータを得るために、全国サーベイランスが必要とされる。
<方法>
我々は、中国の性感染症サーベイランスシステム及び監視サイトネットワークからの症例報告データを収集し評価した。
<結果>
中国において報告された梅毒の全症例発生率は、1993年には100,000例あたり0.2例であったが、2005年には、第一期及び第二期梅毒だけで100,000例あたり5.7例であった。先天的な梅毒の発生率は、1991年は100,000例の出生児あたり0.01症例であったが、2005年には100.000例の出生児あたり19.68例まで、年平均71.9%の割合で大きく増加した。

**使用上の注意記載状況・その他参考事項等**

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びろ過膜処理（ナノフィルトレーション）及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

| 報告企業の意見 | 今後の対応 |
|---|---|
| 中国において梅毒患者が急激に増加しているとの報告である。<br>血漿分画製剤からの梅毒トレポネーマ伝播の事例は報告されていない。梅毒トレポネーマは、低温保管や凍結乾燥、加熱処理により死滅するとされており、またナノフィルトレーションによる除去も容易と考える。そのため、万一原料血漿に梅毒トレポネーマが混入したとしても、製造工程において十分に不活化･除去されると考えている。 | 本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。 |

# Syphilis in China: results of a national surveillance programme

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See Comment page 84

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## Summary

**Background** After a massive syphilis epidemic in the first half of the 20th century, China was able to eliminate this infection for 20 years (1960–80). However, substantial changes in Chinese society have been followed by a resurgent epidemic of sexually transmitted diseases. Sporadic reports have provided clues to the magnitude of the spread of syphilis, but a national surveillance effort is needed to provide data for planning and intervention.

**Methods** We collected and assessed case report data from China's national sexually transmitted disease surveillance system and sentinel site network.

**Findings** In 1993, the reported total rate of cases of syphilis in China was 0·2 cases per 100 000, whereas primary and secondary syphilis alone represented 5·7 cases per 100 000 persons in 2005. The rate of congenital syphilis increased greatly with an average yearly rise of 71·9%, from 0·01 cases per 100 000 livebirths in 1991 to 19·68 cases per 100 000 livebirths in 2005.

**Interpretation** The results suggest that a range of unique biological and social forces are driving the spread of syphilis in China. A national campaign for detection and treatment of syphilis, and a credible prevention strategy, are urgently needed.

## Introduction

Syphilis is a very serious infection that causes acute cutaneous manifestations including genital ulcers, chronic severe and debilitating compromise of the cardiovascular and nervous systems, and serious effects on reproductive and neonatal health.[1] During pregnancy, syphilis infection can lead to spontaneous abortion, congenital deformities, and severe neonatal disease.

Syphilis was first recognised in mainland China in 1505.[2] By the time the Communist party assumed power in 1949, the Chinese people were suffering one of the biggest syphilis epidemics in human history. Surveillance studies in the 1950s showed that the infection was present in as many as 84% of prostitutes and 5% of the general population in some large cities, and 2–3% of rural residents.[3,4]

Mao Zedong and his government prioritised health care, and the control of syphilis and other sexually transmitted diseases (STD) was one of their key aims.[4] In 1952, China developed an unprecedented and unique campaign to eliminate STD. The government instituted mass screening, provided free treatment to infected individuals, and closed brothels.[5,6] This programme seemed to be highly effective, and studies by Chinese and international teams verified the virtual eradication of STDs by the 1960s.[7–10]

However, long-term control of STD in China has proved elusive. Economic and social reforms designed to open China to the world have been successful, but also appear to have laid the foundation for the re-emergence of STD. Increased personal wealth, greater geographical mobility, and more relaxed government control[11] set the stage for the spread of STD. The first cases of syphilis in China after the death of Mao were reported in 1979.[12]

These changes led to the establishment of the Chinese National Center for STD Control in 1986, followed by the launch of a national STD surveillance system by the Ministry of Health in 1987. The purpose of this report is to describe the growth and magnitude of the Chinese syphilis epidemic on the basis of Chinese governmental surveillance data.

## Methods

Surveillance of STD is done through mandatory case-reporting from government facilities including STD clinics, designated provincial dermatovenereology centres, gynaecology and genitourinary clinics, maternity and children's hospitals, and other health providers in all 31 provinces, municipalities, and autonomous regions of the country. Beginning in 2005, syphilis case-reporting moved from paper reports to electronic online submission.

For each syphilis case, doctors or their assistants filled out a standardised STD reporting card with demographic (age in 10 year intervals, sex, home location, marital status, occupation, and education), clinical (diagnostic evidence, onset of symptoms, stage of syphilis, presence of other STD), and epidemiological information (route of acquisition). According to national guidelines,[13] primary syphilis was defined as a clinically compatible case characterised by one or more chancres and inguinal lymphadenopathy, and laboratory confirmation of *Treponema pallidum* in clinical specimens by dark-field microscopy or reactive non-treponemal rapid plasma reagin, or toluidine red unheated serum test and

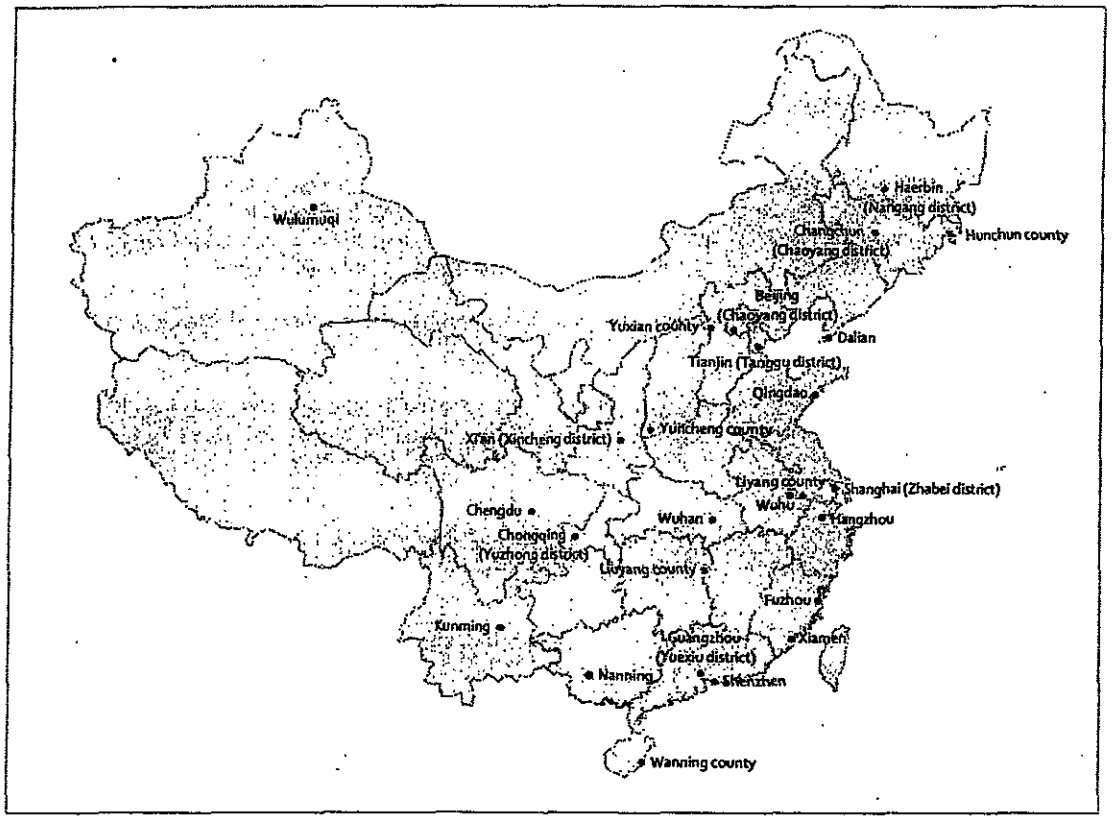


*Figure 1:* Map of 26 national STD sentinel sites in China

treponemal *T pallidum* haemagglutination, or particle agglutination assay for antibody to *T pallidum*; secondary syphilis was defined as a clinically compatible case characterised by maculopapular rash and, in many cases, lymphadenopathy, and laboratory test confirmation as for primary syphilis; latent syphilis was defined as an asymptomatic case with a possible history of infection supported by two consecutive reactive non-treponemal tests (rapid plasma reagin), a reactive treponemal test (particle agglutination assay), and normal cerebrospinal fluid; and tertiary syphilis was defined as a case with a history of primary, secondary, or latent syphilis with clinical manifestations involving the cardiovascular or central nervous system and laboratory confirmation with reactive non-treponemal tests, or cerebrospinal fluid abnormalities characterised by higher than normal amounts of white blood cells or protein. A case of congenital syphilis was defined as a neonate or child younger than 2 years affected by associated signs and symptoms such as rash, hepatosplenomegaly, and lymphadenopathy, with an infected mother, supported by detection of *T pallidum* in lesions by dark-field microscopy or reactive serological test.[14]

To increase the accuracy with which STD incidence could be assessed, a network of 16 sentinel surveillance sites was established in 1987 in areas with high rates of STD. This network was subsequently expanded in 1993 to 26 sites in various cities throughout China. A map of these sentinel sites is shown in figure 1. Each site has a designated organisational centre from either the dermatovenereology institute or Center for Disease Control hospital networks that coordinates mandated STD reporting from all STD service providers in their jurisdiction, which is in most places a city district or in some an entire city. These sites have strengthened management, standardised diagnosis and treatment protocols, and established laboratory quality assurance measures, for STD surveillance. By comparing the number of STD diagnoses recorded in clinic and laboratory registries at sentinel sites with case reports in the surveillance system, twice-yearly quality control studies indicate that sentinel sites report all but less than 5% of newly diagnosed STD cases.

Information from sentinel and non-sentinel sites is collected at the county level and reported quarterly to the next administrative levels until it reaches the National Center for STD Control, where all compiled surveillance data are stored in an electronic database (figure 2).

EpiInfo (Version 1.1, Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to analyse the results in this study.

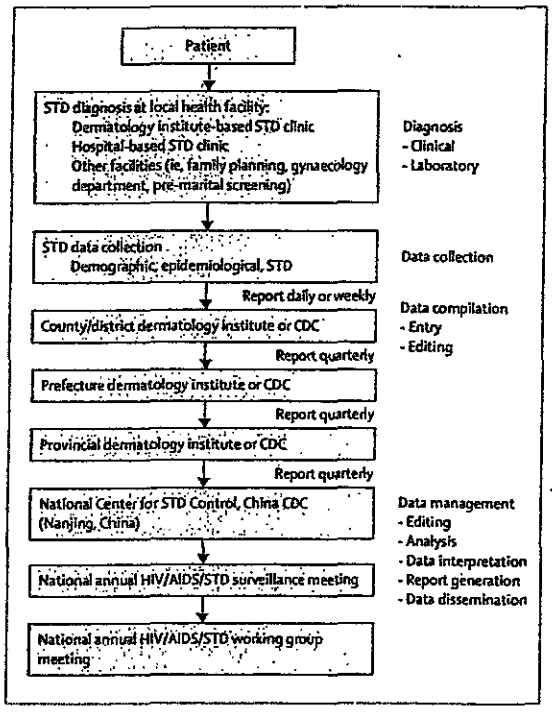


*Figure 2:* Reporting structure of China's nationwide STD surveillance system

Total syphilis incidence refers to the number of new syphilis cases at any stage per 100000 people per year. Because reports based on classification of syphilis into primary, secondary, latent, and tertiary syphilis were not introduced into surveillance reports until 1996, incidence data before this year refer to total syphilis incidence.

The reported incidence of congenital syphilis was calculated by dividing the number of new congenital syphilis cases by the number of livebirths in a specified year. National population and livebirth data were obtained from the National Bureau of Statistics of China.[15] The average yearly rate of increase in incidence was calculated as a geometric mean by use of the formula: (incidence at time n divided by incidence at time 1)^(1/[n–1])–1, where n is defined by the interval number of years.

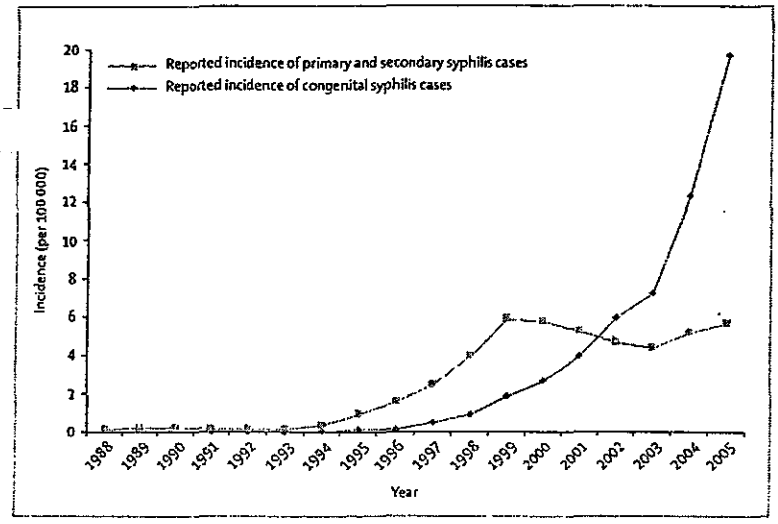


*Figure 3:* Reported incidence of congenital syphilis and cumulative primary and secondary syphilis in China from 1988 to 2005, nationwide STD surveillance system

*Aside from congenital syphilis, classification into different stages of syphilis was introduced in 1996. Thus, before 1996, data points for primary and secondary syphilis show incidence for total syphilis. However, an ad hoc survey of syphilis reports in 38 cities from 1990 to 1994 suggested that reported cases of primary, secondary, early latent, tertiary, and congenital syphilis accounted for 20·2%, 46·5%, 28·1%, 2·1%, and 0·7% of total cases, respectively.

### Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and the team of co-authors had final responsibility for the decision to submit for publication.

## Results

Nationwide surveillance data indicated that after remaining just below 0·2 cases per 100000 people from 1989 to 1993, the total incidence of syphilis increased from 0·17 cases per 100000 in 1993 to 6·5 cases per 100000 in 1999, with incidence of primary and secondary syphilis accounting for 5·85 cases per 100000 (figure 3). After decreasing slightly between 2000 and 2003 (perhaps more a result of administrative changes during that time than a real decline), the incidence of primary and secondary syphilis has increased in the past 2 years to 5·67 cases per 100000 people. The average incidence of primary and secondary syphilis for the years 2000 to 2005 was 5·13 cases per 100000 people per year. Incidence of congenital syphilis had grown at a very rapid rate with an average yearly increase of 71·9%, from 0·01 cases per 100000 livebirths in 1991 to 19·68 cases per 100000 livebirths in 2005 (figure 3). The actual numbers of cases of syphilis classified by the stage of disease are shown in table 1.

More than 70% of reported cases of syphilis in the past 10 years were in people aged 20–49 years. The mean ages were slightly higher in northern and central China than in other regions of the country (table 2). The ratio of male to female individuals with syphilis decreased from 2·0 to 1·0 between 1988 and 2000, and has held steady for the past 5 years.

All China's 2882 counties reported cases of syphilis during the past 5 years. In 2005, the highest rates of total syphilis cases were reported in Shanghai (55·3 cases per 100000 individuals), Zhejiang (35·9 per 100000), and Fujian (26·8 per 100000), which together with Jiangsu (13·0 per 100000) form China's prosperous southeastern seaboard. Beijing municipality had the next highest rate (24·9 per 100000), followed by the southern Zhujiang River Delta (Guangxi, Guangdong, and Hainan provinces), which reported rates of about 14–21 cases per

100 000 individuals. The northeast provinces (Jilin, Liaoning, and Heilongjiang) and the far western provinces of Qinghai and Xinjiang reported rates of 5–12 cases per 100 000 individuals (figure 4).

The 26 sentinel STD sites established in 1993 consistently reported a much higher number of cases than did non-sentinel sites. Incidence of total syphilis cases reported by the enhanced surveillance network rose continuously from 1·4 cases per 100 000 individuals in 1993 to 32·9 per 100 000 in 1999 (figure 5). Incidence fell slightly in 2000 (28·9 cases per 100 000 individuals) was similar in 2001, paralleling the trend reported by the nationwide surveillance system (figure 5). Despite covering a population of only 35–40 million (about 3% of China's population), the 26 sentinel sites reported about 18% of total syphilis cases from 1995 to 2001.

## Discussion

Our report provides a comprehensive view of the re-emergence of syphilis in China. These findings are consistent with reports of the exceptional prevalence of syphilis in high-risk groups in China[16–18] and of the spread of other STD in China,[19] including a nationwide cross-sectional survey that showed a high prevalence of chlamydial infection, especially in urban coastal areas.[20] Worldwide, resurgence of syphilis in China corresponds to epidemics reported in several cities in the UK,[21] Ireland,[22] the Russian Federation states,[23] and the USA.[24]

The incidence of primary and secondary syphilis in 2005 (5·67 cases per 100 000) was substantially higher than that in most developed countries; for example, the USA reported 2·7 cases of primary and secondary syphilis per 100 000 individuals in 2004.[24] Furthermore, the number of cases cited might not accurately reflect the true extent of the epidemic. STDs treated at hospitals, family planning centres, gynaecological clinics, pharmacies, and private practitioners go largely unreported,[25,26] although these venues represent important access points for patients with STDs. A study in Shenzhen and Guangzhou[27] showed that 27% of patients with STDs sought initial care at a site other than a government dermatovenereology centre; in Chengdu[28] and Hangzhou,[29] 18–40% of patients sought care from private doctors and pharmacists. In an internal, unpublished quality-control study done in 1999, STD surveillance officials found that in one province, more than 75% of STD cases were not reported. Private practitioners were the least reliable, reporting only 0·2% of cases, followed by district-level hospitals (7·9%), provincial-level hospitals (15·4%), and city-level hospitals (35·5%).

Surveillance reporting might also be limited by the availability of governmental resources. The 26 sentinel sites reported about 18% of the nation's aggregate syphilis cases, although these sites cover only 3% of the population. Such results suggest that non-sentinel sites are probably not reporting the actual scale of the epidemic, and that improved surveillance would yield a higher overall incidence than reported here.

As an alternative approach we did a systematic review of published work,[17] which suggested the following prevalences of syphilis in specified populations: antenatal women 0·45%; premarital testing 0·66%; voluntary blood donors 0·37%; migrant workers 1·4%; remunerated blood donors 2·86%; possible female sex workers 0·83%; incarcerated female sex workers 12·49%; drug users 6·81%; and men who have sex with men 14·6%.[17] In the past 5–10 years, syphilis rates seem to have increased in all these groups, with the greatest increase in drug users (0·96% per year), incarcerated female sex workers (1·41% per year), and men who have sex with men (4·50% per year).[17] Surveillance data and focused reports from throughout China provide compelling evidence of a substantial and worsening

| | Primary | Secondary | Tertiary | Congenital | Latent | Primary and secondary | Total |
|---|---|---|---|---|---|---|---|
| 1985 | .. | .. | .. | .. | .. | .. | 184 |
| 1986 | .. | .. | .. | .. | .. | .. | 337 |
| 1987 | .. | .. | .. | .. | .. | .. | 819 |
| 1988 | .. | .. | .. | .. | .. | .. | 1387 |
| 1989 | .. | .. | .. | .. | .. | .. | 1976 |
| 1990 | .. | .. | .. | .. | .. | .. | 2574 |
| 1991 | .. | .. | .. | .. | .. | .. | 1870 |
| 1992 | .. | .. | .. | .. | .. | .. | 1997 |
| 1993 | .. | .. | .. | .. | .. | .. | 2016 |
| 1994 | .. | .. | .. | .. | .. | .. | 4591 |
| 1995 | .. | .. | .. | .. | .. | .. | 11336 |
| 1996 | 9036 | 9058 | 80 | 35 | 2548 | 18094 | 20757 |
| 1997 | 14134 | 16085 | 72 | 109 | 3268 | 30219 | 33668 |
| 1998 | 23249 | 25428 | 143 | 185 | 4760 | 48677 | 53765 |
| 1999 | 35805 | 36615 | 285 | 359 | 7342 | 72420 | 80406 |
| 2000 | 36075 | 35424 | 268 | 468 | 7946 | 71499 | 80181 |
| 2001 | 32304 | 33768 | 199 | 677 | 10297 | 66072 | 77245 |
| 2002 | 29978 | 29675 | 277 | 971 | 13428 | 59653 | 74329 |
| 2003 | 30080 | 26415 | 327 | 1155 | 14576 | 56495 | 72553 |
| 2004 | 36420 | 30058 | 501 | 2035 | 19297 | 66478 | 88311 |
| 2005 | 40962 | 33055 | 933 | 3182 | 35556 | 74017 | 113688 |

Table 1: Yearly case reports of syphilis classified by stage, nationwide STD surveillance system

| | Mean age (SD), years |
|---|---|
| North China (Beijing, Tianjin, Hebei, Shanxi, Inner Mongolia) | 39·2 (15·6) |
| Central China (Henan, Hubei, Hunan) | 39·6 (15·5) |
| Northeast China (Liaoning, Heilongjiang, Jilin) | 37·5 (13·6) |
| East China (Shanghai, Zhejiang, Jiangsu, Anhui, Shandong, Jiangxi, Fujian) | 37·9 (13·5) |
| Northwest China (Shaanxi, Gansu, Ningxia, Qinghai, Xinjiang) | 36·6 (15·2) |
| Southwest China (Yunnan, Guizhou, Sichuan, Chongqing, Xizang) | 37·3 (13·9) |
| South China (Guangdong, Guangxi, Hainan) | 36·6 (14·4) |
| Total | 37·6 (14·1) |

Table 2: Age of people with syphilis in 2005 by region

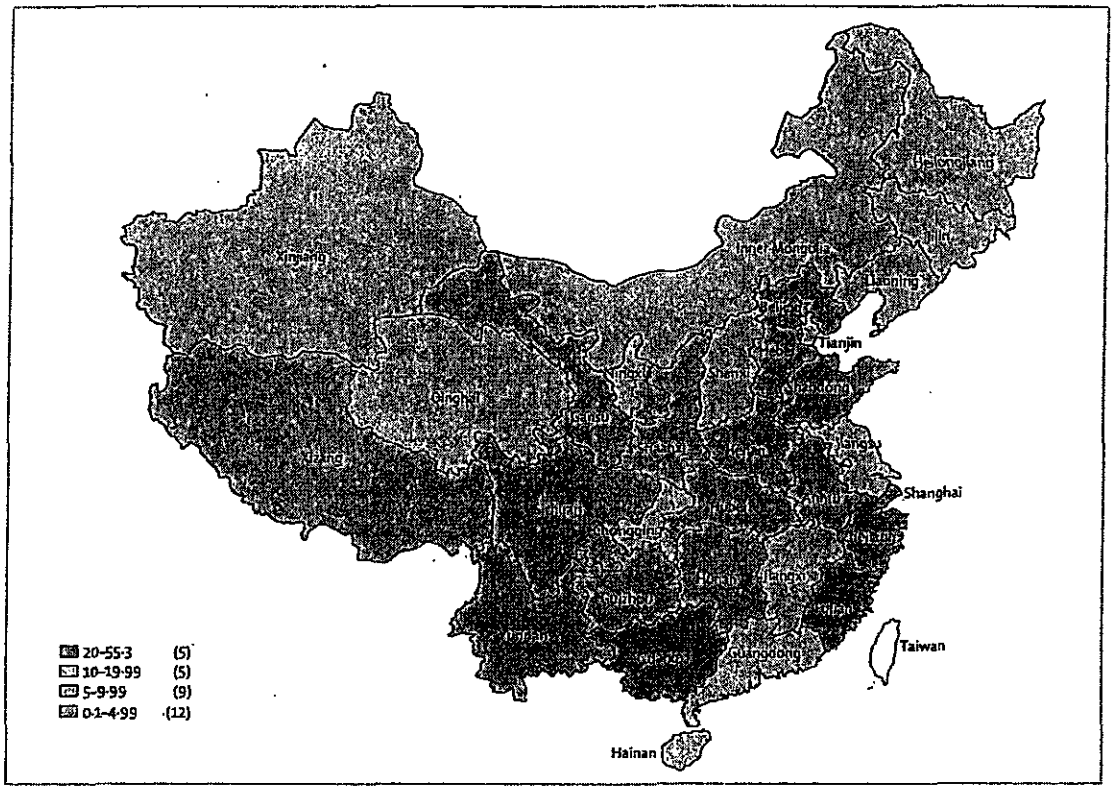


*Figure 4:* Incidence of total syphilis in 2005 by province, nationwide STD surveillance system
Numbers in parentheses show number of provinces in band.

syphilis epidemic in individuals at high risk, and in the general population.

Syphilis can have devastating health consequences, especially if untreated.[1] The increasing privatisation of health care in China has left many people without resources to seek care, particularly for problems like syphilis that on the surface appear to be self-limiting. In a 2004 survey, half the respondents from three provinces reported foregoing health care in the previous year because of its prohibitive cost.[30] This proportion is probably much higher in rural areas, as shown by results from one study done in a rural county outside Beijing where 80% of women with symptoms of STD did not seek health care because of its high cost and their own lack of knowledge about STD.[31]

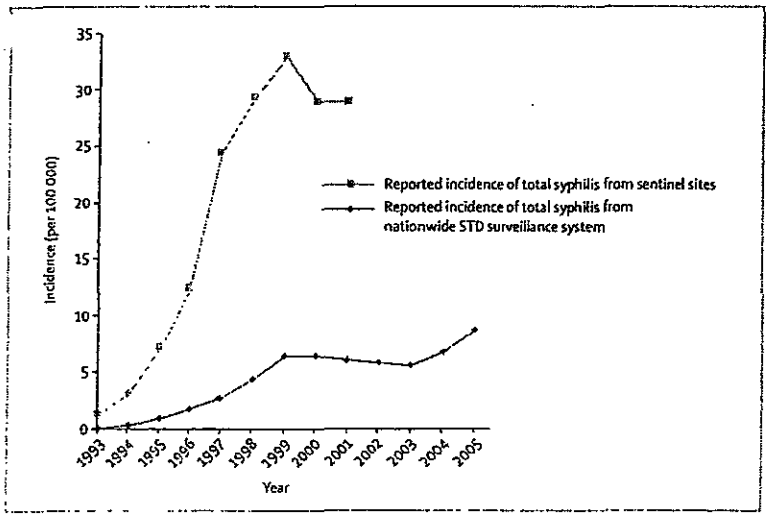


*Figure 5:* Comparison of incidence of syphilis reported by 26 sentinel sites and nationwide STD surveillance system

Obviously, any defects caused by congenital syphilis would be devastating. Additionally, syphilis can contribute to the spread of HIV infection by raising the viral burden in HIV-infected individuals[32] and by increasing susceptibility to infection.[33] Cotransmission of syphilis and HIV has been reported in Pune, India,[34] and syphilis infection in pregnant women might facilitate the vertical transmission of HIV.[35] Although the gravity of syphilis during pregnancy cannot be overstated, detection and treatment of the disease could be overshadowed by focus on the prevention of vertical HIV transmission.[36]

The rapid spread of syphilis in China is probably attributable to a combination of biological and social forces. Grassly and co-workers[37] have argued that the

extent of syphilis observed in a population most closely indicates the degree of herd immunity. In their analysis of changes in syphilis incidence in the USA over the past 50 years, they concluded that 10-year cycles of disease resurgence could result from depletion of individuals with immunity resulting from natural infection.[37] In view of the virtual absence of syphilis in China for 20 years, the general population of young, sexually active individuals would be completely susceptible to this infection.

Social forces could be of even greater importance. Although prostitution in China has an extensive history,[38] strict governmental intervention restricted such practices for nearly 40 years.[6,38] However, economic reforms (so obvious in China's growth and globalisation) have led to income gaps and a cultural climate that favours re-emergence of sex work.[11] A substantial majority of men and a huge migrant population of male workers have created an increasing demand for sex work.[39] It is not a surprise that a high prevalence of syphilis in sex workers has been well documented.[36]

Sexual practices are also changing. Young Chinese people are increasingly likely to experiment with sex at earlier ages and before marriage.[40] Additionally, reduced governmental emphasis on public-health services, increasing costs of individual health care, and reduced availability of health insurance[41] might have contributed to reduced recognition and treatment of syphilis.

Clearly, China must attack the spread of STD and especially syphilis. One key component of this intervention is affordable syphilis screening, the tool that proved so powerful in the earliest days of Mao's government.[4] The southern city of Shenzhen has pioneered free syphilis screening for all pregnant women, a programme that has been shown to be cost effective and sustainable.[42] Further efforts should also be directed at groups in which high syphilis prevalence has been documented, such as men who have sex with men[43] and female sex workers.[44] Thousands of health centres in China already offer free voluntary counselling and testing for HIV, and additional testing for syphilis in these centres would expand coverage of syphilis screening and help to integrate STD and HIV prevention efforts. However, these strategies will need much support from local and national leaders, as part of the overall urban and rural health reforms.[45]

STD prevention programmes need to be scaled-up for a mass audience. Preliminary results from the 100% condom use programmes for sex workers promoted in Jiangsu, Hubei, Hunan, Guangxi, and Hainan provinces are favourable, with sharp drops in the prevalence of syphilis in female sex workers.[46] These targeted interventions have been complemented by mass education campaigns directed at younger people.

China has recently shown a great interest in the control of infectious diseases. Although the country was perhaps slow to recognise the threat of HIV, this infection is now a key governmental focus for which many active programmes are being developed, including needle exchange and methadone schemes for intravenous drug users, and free medical care for those who are infected with HIV. The resources put into place to control severe acute respiratory syndrome (SARS)[47] and avian influenza[48] are also impressive.

The spread of syphilis in China has been insidious, and has only recently attracted the attention it deserves. Initial efforts to respond to this epidemic include the pilot screening and prevention programmes and national consultative meetings in 2005 and 2006 that have generated a response strategy and helped to place syphilis on the national agenda. China has shown once before that the control of syphilis is possible,[9] and we expect that a new national campaign, although challenging, will show benefit.

**Contributors**

This project was designed and planned by X-S Chen and M S Cohen. Z-Q Chen and G-C Zhang coordinated data collection and manuscript preparation. Data were collected by X-D Gong, C Lin, X Gao, G-J Liang, and X-L Yue, and analysed by X-D Gong, C Lin, X Gao, X-S Chen, and M S Cohen. C Lin, X Gao, X-S Chen, and M S Cohen drafted and edited the manuscript.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgments**

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# B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料Eにおいて集積報告を行っているため、添付していない）。

# 感染症定期報告の報告状況(2007/3/1～2007/5/31)

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 措置 |
|---|---|---|---|---|---|---|---|---|---|---|
| 60232 | 2007/03/15 | 日本メジフィジックス | 放射性医薬品基準テクネチウム大凝集人血清アルブミン(99mTc)注射液 | テクネチウム大凝集人血清アルブミン(99mTc) | 生物学的製剤基準人血清アルブミン | 日本 | 有効成分 | 無 | 無 | 無 |
| 60233 | 2007/03/20 | 化学及血清療法研究所 | 人免疫グロブリン<br>ヒスタミン加人免疫グロブリン製剤 | 免疫グロブリン | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 60234 | 2007/03/23 | ベネシス | ①②③人血清アルブミン<br>④乾燥濃縮人血液凝固第Ⅷ因子<br>⑤乾燥濃縮人血液凝固第Ⅸ因子 | 人血清アルブミン | 人血液 | ①②④⑤日本、③米国 | ①②③有効成分、④⑤添加物 | 有 | 無 | 無 |
| 60235 | 2007/03/23 | ベネシス | 乾燥濃縮人血液凝固第Ⅷ因子 | 血液凝固第Ⅷ因子 | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
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| 60237 | 2007/03/27 | 日本製薬 | 乾燥ポリエチレングリコール処理人免疫グロブリン | ポリエチレングリコール処理人免疫グロブリンG | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
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| 60244 | 2007/03/29 | 化学及血清療法研究所 | フィブリノゲン加第ＸⅢ因子 | 人血液凝固第ＸⅢ因子 | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 60245 | 2007/03/29 | 化学及血清療法研究所 | ①フィブリノゲン加第ＸⅢ因子<br>②乾燥濃縮人活性化プロテインC<br>③トロンビン | トロンビン | ヒト血液 | 日本 | ①③有効成分、②製造工程 | 有 | 無 | 無 |

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 適正使 |
|---|---|---|---|---|---|---|---|---|---|---|
| 60246 | 2007/03/29 | 化学及血清療法研究所 | ①ボルヒール<br>②乾燥濃縮人活性化プロテインC<br>③乾燥濃縮人血液凝固第Ⅸ因子<br>④、⑧乾燥スルホ化人免疫グロブリン<br>⑤、⑥、⑦、⑩、⑪人血清アルブミン<br>⑨乾燥濃縮人血液凝固第Ⅷ因子 | 人血清アルブミン | ヒト血液 | 日本 | ⑤～⑦、⑩、⑪有効成分、①～④、⑧、⑨ | 有 | 無 | 無 |
| 60247 | 2007/03/29 | 化学及血清療法研究 | フィブリノゲン加第ⅩⅢ因子 | アプロチニン | ウシ肺臓 | ウルグアイ | 有効成分 | 有 | 無 | 無 |
| 60248 | 2007/03/30 | 日本赤十字社 | （製造承認書に記載なし） | 合成血 | 人血液 | 日本 | 有効成分 | 有 | 無 | 有 |
| 60249 | 2007/03/30 | バクスター | 乾燥濃縮人血液凝固第Ⅷ因子 | 乾燥人血液凝固第Ⅷ因子 | 人血漿 | 米国 | 有効成分 | 有 | 有 | 無 |
| 60250 | 2007/03/30 | バクスター | 乾燥濃縮人血液凝固第Ⅷ因子 | 人血清アルブミン | 人血漿 | 米国 | 添加物 | 有 | 有 | 無 |
| 60251 | 2007/03/30 | バクスター | ルリオクトコグ　アルファ(遺伝子組換え) | ルリオクトコグ　アルファ(遺伝子組換え) | 遺伝子組換えチャイニーズハムスター卵巣細胞株 | 該当なし | 有効成分 | 有 | 無 | 無 |
| 60252 | 2007/03/30 | バクスター | 乾燥人血液凝固因子抗体迂回活性複合体 | 乾燥人血液凝固因子抗体迂回活性複合体 | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |
| 60253 | 2007/03/30 | バクスター | 乾燥人血液凝固第Ⅸ因子複合体 | 乾燥人血液凝固第Ⅸ因子複合体 | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |
| 60254 | 2007/03/30 | バクスター | 乾燥人血液凝固第Ⅸ因子複合体 | ヘパリンナトリウム | ブタ胃 | 中国 | 添加物 | 無 | 無 | 無 |
| 60255 | 2007/03/30 | 日本赤十字社 | 人血清アルブミン | 人血清アルブミン | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70001 | 2007/04/10 | 化学及血清療法研究 | 乾燥濃縮人活性化プロテインC | プロテインC | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70002 | 2007/04/10 | 化学及血清療法研究 | 乾燥濃縮人活性化プロテインC<br>乾燥濃縮人血液凝固第Ⅸ因子 | マウス由来モノクローナル抗体 | マウス脾臓 | 日本 | 製造工程 | 無 | 無 | 無 |
| 70003 | 2007/04/13 | 日本メジフィジックス | 放射性医薬品基準人血清アルブミン五酢酸テクネチウム(99mTc)注射液 | 人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc) | 生物学的製剤基準人血清アルブミン | 日本 | 有効成分 | 有 | 無 | 無 |
| 70004 | 2007/04/23 | ベネシス | 乾燥濃縮人血液凝固第Ⅸ因子 | ヤギIgG | ヤギ血液 | オーストラリア | 製造工程 | 無 | 無 | 無 |
| 70005 | 2007/04/23 | ベネシス | 乾燥濃縮人血液凝固第Ⅸ因子 | マウスモノクローナル抗体 | マウス脾臓細胞と骨髄腫細胞のハイブリドーマ | イギリス | 製造工程 | 無 | 無 | 無 |
| 70006 | 2007/04/23 | ベネシス | 乾燥抗HBs人免疫グロブリン<br>ポリエチレングリコール処理抗HBs人免疫グロブリン | 抗HBs抗体 | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70007 | 2007/04/24 | CSLベーリング | | ヒトアルブミン | ヒト血液 | 米国、ドイツ、オースト | 添加物 | 有 | 無 | 無 |

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 適正使 |
|---|---|---|---|---|---|---|---|---|---|---|
| 70008 | 2007/04/24 | CSLベーリング | | ヒトフィブリノゲン | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 無 | 無 |
| 70009 | 2007/04/24 | CSLベーリング | | アンチトロンビンⅢ | ヒト血液 | 米国、ドイツ、オースト | 製造工程 | 有 | 無 | 無 |
| 70010 | 2007/04/24 | CSLベーリング | | トロンビン画分 | ウシ血液 | ニュージーラン | 有効成分 | 有 | 無 | 無 |
| 70011 | 2007/04/24 | CSLベーリング | | アプロチニン | ウシ肺 | ウルグアイ、ニュー | 有効成分 | 有 | 無 | 無 |
| 70012 | 2007/04/24 | CSLベーリング | | ヘパリン | ブタ腸粘膜 | 中国 | 製造工程 | 無 | 無 | 無 |
| 70013 | 2007/04/24 | CSLベーリング | | トロンボプラスチン | ウサギ脳 | ニュージーラン | 製造工程 | 無 | 無 | 無 |
| 70014 | 2007/04/24 | CSLベーリング | | ウマコラーゲン | ウマアキレス腱 | フランス、ドイツ、ベルギー、イタリア | 支持体 | 無 | 無 | 無 |
| 70015 | 2007/04/25 | 日本製薬 | 乾燥抗HBs人免疫グロブリン | 抗HBs抗体 | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70016 | 2007/04/25 | 日本製薬 | 乾燥抗破傷風人免疫グロブリン | 破傷風抗毒素 | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70017 | 2007/04/25 | バイエル薬品 | pH4処理酸性人免疫グロブリン | 人免疫グロブリンG | ヒト血液 | 米国 | 有効成分 | 有 | 有 | 無 |
| 70018 | 2007/04/25 | バイエル薬品 | オクトコグ　アルファ(遺伝子組換え) | ヒトトランスフェリン | ヒト血液 | 米国 | 製造工程 | 有 | 無 | 無 |
| 70019 | 2007/04/25 | バイエル薬品 | ①加熱人血漿たん白<br>②、③オクトコグ　アルファ(遺伝子組換え) | 加熱人血漿たん白 | ヒト血液 | 米国 | ①有効成分<br>②、③製造工程 | 有 | 無 | 無 |
| 70020 | 2007/04/25 | バイエル薬品 | ①人血清アルブミン<br>②オクトコグ　アルファ(遺伝子組換え) | 人血清アルブミン | ヒト血液 | 米国 | ①有効成分<br>②製造工程 | 有 | 無 | 無 |
| 70021 | 2007/04/25 | バイエル薬品 | オクトコグ　アルファ(遺伝子組換え) | ウシインスリン | ウシ膵臓 | 米国 | 製造工程 | 有 | 無 | 無 |
| 70022 | 2007/04/26 | 化学及血清療法研究 | 抗HBs人免疫グロブリン | 抗HBs人免疫グロブリン | ヒト血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70023 | 2007/04/26 | 日本赤十字社 | 乾燥濃縮人血液凝固第Ⅷ因子 | 乾燥濃縮人血液凝固第Ⅷ因子 | 人血液 | 日本 | 有効成分 | 有 | 無 | 有 |
| 70024 | 2007/04/26 | 日本赤十字社 | 乾燥濃縮人血液凝固第Ⅷ因子 | 人血清アルブミン | 人血液 | 日本 | 添加物 | 有 | 無 | 有 |
| 70025 | 2007/04/26 | 日本赤十字社 | 人免疫グロブリン | 人免疫グロブリン | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70026 | 2007/04/26 | 日本赤十字社 | pH4処理酸性人免疫グロブリン | pH4処理酸性人免疫グロブリン | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 適正使 |
|---|---|---|---|---|---|---|---|---|---|---|
| 70027 | 2007/04/26 | バクスター | 人血清アルブミン | 人血清アルブミン | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70028 | 2007/04/26 | CSLベーリング | フィブリノゲン加第XIII因子 | トロンビン末 | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 無 | 無 |
| 70029 | 2007/04/26 | CSLベーリング | フィブリノゲン加第XIII因子 | フィブリノゲン | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 無 | 無 |
| 70030 | 2007/04/26 | CSLベーリング | 抗破傷風人免疫グロブリン | 破傷風抗毒素 | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 無 | 無 |
| 70031 | 2007/04/26 | CSLベーリング | フィブリノゲン加第XIII因子 | アンチトロンビン | ヒト血液 | 米国、ドイツ、オースト | 製造工程 | 有 | 無 | 無 |
| 70032 | 2007/04/26 | CSLベーリング | フィブリノゲン加第XIII因子<br>人血液凝固第XIII因子 | 人血液凝固第XIII因子 | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 無 | 無 |
| 70033 | 2007/04/26 | CSLベーリング | 人免疫グロブリン | 人免疫グロブリン | ヒト血液 | 米国、ドイツ、オースト | 有効成分 | 有 | 有 | 無 |
| 70034 | 2007/04/27 | 日本赤十字社 | 新鮮凍結人血漿 | 新鮮凍結人血漿 | 人血液 | 日本 | 有効成分 | 有 | 有 | 有 |
| 70035 | 2007/04/27 | 日本赤十字社 | 人血小板濃厚液 | 人血小板濃厚液 | 人血液 | 日本 | 有効成分 | 有 | 有 | 有 |
| 70036 | 2007/04/27 | 日本赤十字社 | 洗浄人赤血球浮遊液 | 洗浄人赤血球浮遊液 | 人血液 | 日本 | 有効成分 | 有 | 有 | 有 |
| 70037 | 2007/04/27 | 日本赤十字社 | 白血球除去人赤血球浮遊液 | 白血球除去人赤血球浮遊液 | 人血液 | 日本 | 有効成分 | 有 | 有 | 有 |
| 70038 | 2007/05/14 | バクスター | 人血清アルブミン | 人血清アルブミン | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70039 | 2007/05/14 | バクスター | 人血清アルブミン | ヘパリンナトリウム | ブタ腸 | 中国 | 製造工程 | 無 | 無 | 無 |
| 70040 | 2007/05/14 | バクスター | 活性化プロトロンビン複合体濃縮製剤 | ヘパリンナトリウム | ブタ腸 | 該当なし | 添加物 | 無 | 無 | 無 |
| 70041 | 2007/05/14 | バクスター | 活性化プロトロンビン複合体濃縮製剤 | 乾燥人血液凝固第Ⅷ因子阻害物質補正活性複合体 | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70042 | 2007/05/14 | バクスター | 該当なし | ヒト凝固性たん白質(フィブリノゲン加第XⅢ因子) | 人血漿 | 米国 | 有効成分 | 有 | 有 | 無 |
| 70043 | 2007/05/14 | バクスター | 該当なし | 人血清アルブミン | 人血漿 | 米国 | 添加物 | 有 | 有 | 無 |
| 70044 | 2007/05/14 | バクスター | 該当なし | トロンビン | 人血漿 | 米国 | 有効成分 | 有 | 有 | 無 |
| 70045 | 2007/05/14 | バクスター | 乾燥人血液凝固第Ⅸ因子複合体 | ヘパリンナトリウム | ブタ腸 | 中国 | 添加物 | 無 | 無 | 無 |
| 70046 | 2007/05/14 | バクスター | 乾燥人血液凝固第Ⅸ因子複合体 | 乾燥人血液凝固第Ⅸ因子複合体 | 人血漿 | 米国 | 有効成分 | 有 | 無 | 無 |

| 血対ID | 受理日 | 報告者名 | 一般名 | 生物由来成分名 | 原材料名 | 原産国 | 含有区分 | 文献 | 症例 | 過正使 |
|---|---|---|---|---|---|---|---|---|---|---|
| 70047 | 2007/05/21 | 富士フイルムRIファーマ | テクネチウム人血清アルブミン(99mTc) | テクネチウム人血清アルブミン(99mTc) | ヒト血液 | 日本 | 有効成分 | 無 | 無 | 無 |
| 70048 | 2007/05/22 | ベネシス | 乾燥人フィブリノゲン | 凝固性たん白質 | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70049 | 2007/05/22 | ベネシス | 乾燥濃縮人アンチトロンビンIII | 人アンチトロンビンIII | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70050 | 2007/05/22 | ベネシス | トロンビン | トロンビン | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70051 | 2007/05/22 | ベネシス | 乾燥抗D(Rho)人免疫グロブリン | 抗D(Rho)抗体含有人免疫グロブリンG | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70052 | 2007/05/22 | ベネシス | 乾燥濃縮人血液凝固第IX因子 | 血液凝固第IX因子 | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70053 | 2007/05/22 | 化学及血清療法研究所 | 乾燥ペプシン処理人免疫グロブリン | ペプシン処理人免疫グロブリンG分屑 | ヒト血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70054 | 2007/05/22 | 化学及血清療法研究所 | 乾燥ペプシン処理人免疫グロブリン | ペプシン | ブタ胃粘膜 | 米国、カナダ | 製造工程 | 無 | 無 | 無 |
| 70055 | 2007/05/24 | 日本製薬 | 人免疫グロブリン | 免疫グロブリンG | 人血液 | 日本 | 有効成分 | 有 | 無 | 無 |
| 70056 | 2007/05/24 | 日本製薬 | 乾燥抗D(Rho)人免疫グロブリン | 抗D(Rho)抗体 | 人血液 | 米国 | 有効成分 | 有 | 無 | 無 |
| 70057 | 2007/05/29 | 日本赤十字社 | 解凍人赤血球濃厚液 | 解凍人赤血球濃厚液 | 人血液 | 日本 | 有効成分 | 有 | 無 | 有 |
| 70058 | 2007/05/29 | CSLベーリング | 乾燥pH4処理人免疫グロブリン | 人免疫グロブリンG | ヒト血液 | ドイツ | 有効成分 | 有 | 有 | 無 |

別紙様式第４

感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第３回 | 3-1 | 感染症および寄生虫症 | ロタウイルス胃腸炎 | 日本 | 女 | 1 | 2004年４月20日 | 軽快 | 症例報告 | 当該製品 | |

| | | | | |
|---|---|---|---|---|
| 60236 | 2007/03/23 | ベネシス | ①②ポリエチレングリコール処理人免疫グロブリン<br>③人免疫グロブリン | 人免疫グロブリンG |



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別紙様式第４

感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第８回 | 8-1 | 全身障害および投与局所様態 | 発熱 | 日本 | 男 | 11ヵ月 | 2006年７月８日 | 回復 | 症例報告 | 当該製品 | 未完了報告①：2006年９月19日<br>未完了報告②：2006年10月３日<br>未完了報告③：2006年10月18日<br>識別番号：A-06000119<br>ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。 |
| | | 皮膚および皮下組織障害 | 発疹 | | | | 2006年７月11日 | 回復 | | | |
| | | 肝胆道系障害 | 肝障害 | | | | 2006年７月10日 | 回復 | | | |
| | 8-2 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 女 | 6 | 2006年11月13日 | 回復 | 症例報告 | 当該製品 | |
| | 8-3 | 皮膚および皮下組織障害 | 全身紅斑 | 日本 | 男 | 1 | 2007年１月２日 | 回復 | 症例報告 | 当該製品 | 未完了報告：2007年１月31日<br>識別番号：A-06000214<br>ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。<br>（その後の調査でウイルス感染の可能性が否定されたため、2007年２月15日に取り下げ報告を行った。） |
| 第７回 | 6-1 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 男 | 1 | 2005年10月11日 | 回復 | 症例報告 | 当該製品 | 取下げ報告：2006年３月24日<br>識別番号：A-05000194 |
| 第６回 | 6-1 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 男 | 1 | 2005年10月11日 | 回復 | 症例報告 | 当該製品 | 未完了報告①：2005年11月14日<br>未完了報告②：2005年12月１日<br>識別番号：A-05000194 |
| | 5-1 | 肝胆道系障害 | 肝機能障害 | 日本 | 男 | 11ヵ月 | 2005年４月５日 | 回復 | 症例報告 | 当該製品 | 完了報告：2005年９月26日<br>識別番号：A-05000095<br>第５回報告の「ウイルス性肝炎」から副作用・感染症名が「肝機能障害」に変更になった。 |
| 第５回 | 5-1 | 感染症および寄生虫症 | ウイルス性肝炎 | 日本 | 男 | 11ヵ月 | 2005年４月５日 | 回復 | 症例報告 | 当該製品 | 未完了報告：2005年７月27日<br>識別番号：A-05000095 |

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別紙様式第4

別紙(5)

感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発生国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 感染症および寄生虫症 | B型肝炎 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 1 | 臨床検査 | B型肝炎表面抗原陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 1 | 臨床検査 | 抗HBs抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 1 | 臨床検査 | 抗HBcIgG抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 1 | 臨床検査 | 抗HBe抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 男 | 37 | 不明 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 3 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　提出日2006年12月7日 |
| | 3 | 臨床検査 | C型肝炎抗体陽性 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　提出日2006年12月7日 |
| | 3 | 臨床検査 | C型肝炎RNA陽性 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　提出日2006年12月7日 |
| | 3 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　追加報告提出日2006年12月20日 |
| | 3 | 臨床検査 | C型肝炎抗体陽性 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　追加報告提出日2006年12月20日 |
| | 3 | 臨床検査 | C型肝炎RNA陽性 | ドイツ | 女 | 41 | 2006/11/21 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号3-06000029　追加報告提出日2006年12月20日 |
| 第7回 | 1 | 感染症および寄生虫症 | C型肝炎 | 米国 | 不明 | 不明 | 2006年 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号:3-06000004 提出日:2006/4/25 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号:3-06000004 追加報告提出日:2006/4/27 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号:3-06000004 追加報告提出日:2006/5/18 |

| | | | | |
|---|---|---|---|---|
| 60242 | 2007/03/28 | ZLBベーリング | ①～③人血清アルブミン<br>④人血液凝固第ＸⅢ因子<br>⑤⑥フィブリノゲン加第ＸⅢ因子 | 人血清アルブミン |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 感染症の種類 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA Ver. | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第8回 | 7-012 | 臨床検査 | C型肝炎 | アルゼンチン | 男性 | 11 | 2006/5/2 | 不明 | 症例報告 | 外国製品 | 06000019 | 2006/9/1 | 9.0 | 第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告 |
| 第8回 | 7-012 | 臨床検査 | C型肝炎 | アルゼンチン | 男性 | 11 | 2006/5/2 | 不明 | 症例報告 | 外国製品 | 06000019 | 2006/9/25 | 9.0 | 第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告 |
| 第8回 | 7-012 | 臨床検査 | ウイルス負荷増加 | アルゼンチン | 男性 | 11 | 2006/5/2 | 不明 | 症例報告 | 外国製品 | 06000019 | 2006/9/25 | 9.0 | 第8回症例番号7-012は第7回症例番号7-012において報告したものの追加報告 |
| 第7回 | 7-022 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000648 | 2006/3/3 | 8.1 | |
| 第7回 | 7-007 | 感染症および寄生虫症 | A型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000013 | 2006/5/15 | 9.0 | |
| 第7回 | 7-023 | 臨床検査 | A型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000649 | 2006/3/3 | 8.1 | |
| 第7回 | 7-021 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000647 | 2006/3/3 | 8.1 | |
| 第7回 | 7-001 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 24 | 不明 | 不明 | 症例報告 | 外国製品 | 06000007 | 2006/5/1 | 9.0 | |
| 第7回 | 7-002 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 9 | 不明 | 不明 | 症例報告 | 外国製品 | 06000009 | 2006/5/10 | 9.0 | |
| 第7回 | 7-008 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000011 | 2006/5/10 | 9.0 | |
| 第7回 | 7-007 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000013 | 2006/5/15 | 9.0 | |
| 第7回 | 7-006 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000018 | 2006/5/22 | 9.0 | |
| 第7回 | 7-023 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000649 | 2006/3/3 | 8.1 | |
| 第7回 | 7-024 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000650 | 2006/3/3 | 8.1 | |
| 第7回 | 7-011 | 感染症および寄生虫症 | C型肝炎 | 台湾 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000635 | 2006/3/2 | 8.1 | |
| 第7回 | 7-009 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000637 | 2006/3/3 | 8.1 | |
| 第7回 | 7-013 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000638 | 2006/3/3 | 8.1 | |
| 第7回 | 7-014 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000639 | 2006/3/3 | 8.1 | |
| 第7回 | 7-015 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000640 | 2006/3/3 | 8.1 | |
| 第7回 | 7-016 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000641 | 2006/3/3 | 8.1 | |
| 第7回 | 7-017 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000642 | 2006/3/3 | 8.1 | |
| 第7回 | 7-018 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000643 | 2006/3/3 | 8.1 | |
| 第7回 | 7-019 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000644 | 2006/3/3 | 8.1 | |
| 第7回 | 7-020 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000645 | 2006/3/3 | 8.1 | |
| 第7回 | 7-004 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000646 | 2006/3/3 | 8.1 | |
| 第7回 | 7-022 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000648 | 2006/3/3 | 8.1 | |
| 第7回 | 7-059 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 55 | 不明 | 不明 | 症例報告 | 外国製品 | 06000006 | 2006/5/1 | 9.0 | |
| 第7回 | 7-001 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 24 | 不明 | 不明 | 症例報告 | 外国製品 | 06000007 | 2006/5/1 | 9.0 | |
| 第7回 | 7-060 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000008 | 2006/5/1 | 9.0 | |
| 第7回 | 7-002 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 9 | 不明 | 不明 | 症例報告 | 外国製品 | 06000009 | 2006/5/10 | 9.0 | |
| 第7回 | 7-003 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000010 | 2006/5/10 | 9.0 | |
| 第7回 | 7-008 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000011 | 2006/5/10 | 9.0 | |
| 第7回 | 7-007 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000013 | 2006/5/15 | 9.0 | |
| 第7回 | 7-061 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000014 | 2006/5/15 | 9.0 | |
| 第7回 | 7-010 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 06000015 | 2006/5/15 | 9.0 | |
| 第7回 | 7-005 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000016 | 2006/5/15 | 9.0 | |
| 第7回 | 7-062 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000017 | 2006/5/15 | 9.0 | |
| 第7回 | 7-006 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 06000018 | 2006/5/22 | 9.0 | |
| 第7回 | 7-012 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 11 | 2006/5/2 | 不明 | 症例報告 | 外国製品 | 06000019 | 2006/6/16 | 9.0 | |
| 第7回 | 5-130 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000065 | 2006/3/30 | 9.0 | 第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄 |
| 第7回 | 5-139 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000104 | 2006/3/2 | 8.1 | 第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 感染症の種類 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 備考 識別番号 | 報告日 | MedDRA(Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第7回 | 7-023 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000649 | 2006/3/3 | 8.1 | |
| 第7回 | 7-024 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000650 | 2006/3/3 | 8.1 | |
| 第7回 | 7-025 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000651 | 2006/3/3 | 8.1 | |
| 第7回 | 7-026 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000652 | 2006/3/3 | 8.1 | |
| 第7回 | 7-027 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000653 | 2006/3/3 | 8.1 | |
| 第7回 | 7-028 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000654 | 2006/3/3 | 8.1 | |
| 第7回 | 7-029 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000655 | 2006/3/3 | 8.1 | |
| 第7回 | 7-030 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000656 | 2006/3/3 | 8.1 | |
| 第7回 | 7-031 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000657 | 2006/3/3 | 8.1 | |
| 第7回 | 7-032 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000658 | 2006/3/3 | 8.1 | |
| 第7回 | 7-033 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000659 | 2006/3/3 | 8.1 | |
| 第7回 | 7-034 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000660 | 2006/3/3 | 8.1 | |
| 第7回 | 7-035 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000661 | 2006/3/13 | 8.1 | |
| 第7回 | 7-036 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000662 | 2006/3/13 | 8.1 | |
| 第7回 | 7-037 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000663 | 2006/3/13 | 8.1 | |
| 第7回 | 7-038 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000664 | 2006/3/13 | 8.1 | |
| 第7回 | 7-039 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000665 | 2006/3/13 | 8.1 | |
| 第7回 | 7-040 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000666 | 2006/3/13 | 8.1 | |
| 第7回 | 7-041 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000667 | 2006/3/13 | 8.1 | |
| 第7回 | 7-042 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000668 | 2006/3/13 | 8.1 | |
| 第7回 | 7-043 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000669 | 2006/3/13 | 8.1 | |
| 第7回 | 7-044 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000670 | 2006/3/13 | 8.1 | |
| 第7回 | 7-045 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000671 | 2006/3/13 | 8.1 | |
| 第7回 | 7-046 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000672 | 2006/3/13 | 8.1 | |
| 第7回 | 7-047 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000673 | 2006/3/13 | 8.1 | |
| 第7回 | 7-048 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000674 | 2006/3/13 | 8.1 | |
| 第7回 | 7-049 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000675 | 2006/3/13 | 8.1 | |
| 第7回 | 7-050 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000676 | 2006/3/13 | 8.1 | |
| 第7回 | 7-051 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000677 | 2006/3/13 | 8.1 | |
| 第7回 | 7-052 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000678 | 2006/3/13 | 8.1 | |
| 第7回 | 7-053 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000679 | 2006/3/13 | 8.1 | |
| 第7回 | 7-054 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000680 | 2006/3/13 | 8.1 | |
| 第7回 | 7-055 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000681 | 2006/3/13 | 8.1 | |
| 第7回 | 7-056 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000682 | 2006/3/13 | 8.1 | |
| 第7回 | 7-057 | 臨床検査 | C型肝炎ウイルス | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000683 | 2006/3/13 | 8.1 | |
| 第7回 | 7-058 | 臨床検査 | C型肝炎ウイルス | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000684 | 2006/3/13 | 8.1 | |
| 第7回 | 5-139 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 1985 | 死亡 | 症例報告 | 外国製品 | 05000104 | 2006/3/2 | 8.1 | 第7回症例番号5-139は第5回症例番号5-139において報告したものの追加報告 |
| 第7回 | 7-001 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 24 | 1985 | 不明 | 症例報告 | 外国製品 | 06000007 | 2006/5/1 | 9.0 | |
| 第7回 | 7-002 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 9 | 1985 | 不明 | 症例報告 | 外国製品 | 06000009 | 2006/5/10 | 9.0 | |
| 第7回 | 7-003 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1985/10/4 | 不明 | 症例報告 | 外国製品 | 06000010 | 2006/5/10 | 9.0 | |
| 第7回 | 7-004 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000646 | 2006/3/3 | 8.1 | |
| 第7回 | 7-005 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 06000016 | 2006/5/15 | 9.0 | |
| 第7回 | 7-006 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 06000018 | 2006/5/22 | 9.0 | |
| 第7回 | 7-007 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1986/3 | 不明 | 症例報告 | 外国製品 | 06000013 | 2006/5/15 | 9.0 | |
| 第7回 | 7-008 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1986/4/9 | 不明 | 症例報告 | 外国製品 | 06000011 | 2006/5/10 | 9.0 | |
| 第7回 | 7-009 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1988 | 不明 | 症例報告 | 外国製品 | 05000637 | 2006/3/3 | 8.1 | |
| 第7回 | 7-010 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 不明 | 1988/5 | 死亡 | 症例報告 | 外国製品 | 06000015 | 2006/5/15 | 9.0 | |
| 第7回 | 7-011 | 感染症および寄生虫症 | HIV感染 | 台湾 | 男性 | 不明 | 1997/4/17 | 不明 | 症例報告 | 外国製品 | 05000635 | 2006/3/2 | 8.1 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 |区分 | 識別番号 | 報告日 | 備考 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 疾患別大分類 | 基本語（PT） | | | | | | | | | | MedDRA（Ver.） | |
| 第7回 | 5-130 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000065 | 2006/3/30 | 9.0 | 第7回症例番号5-130は第5回症例番号5-130と重複症例のため報告破棄 |
| 第7回 | 7-013 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000638 | 2006/3/3 | 8.1 | |
| 第7回 | 7-020 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000645 | 2006/3/3 | 8.1 | |
| 第7回 | 7-021 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000647 | 2006/3/3 | 8.1 | |
| 第7回 | 7-044 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000670 | 2006/3/13 | 8.1 | |
| 第7回 | 7-045 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000671 | 2006/3/13 | 8.1 | |
| 第7回 | 7-046 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000672 | 2006/3/13 | 8.1 | |
| 第7回 | 7-047 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000673 | 2006/3/13 | 8.1 | |
| 第7回 | 7-048 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000674 | 2006/3/13 | 8.1 | |
| 第7回 | 7-049 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000675 | 2006/3/13 | 8.1 | |
| 第7回 | 7-057 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000683 | 2006/3/13 | 8.1 | |
| 第7回 | 7-058 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000684 | 2006/3/13 | 8.1 | |
| 第6回 | 6-126 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 回復 | 症例報告 | 外国製品 | 05000534 | 2006/2/8 | 8.1 | |
| 第6回 | 6-148 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 回復 | 症例報告 | 外国製品 | 05000559 | 2006/2/13 | 8.1 | |
| 第6回 | 6-153 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000565 | 2006/2/13 | 8.1 | |
| 第6回 | 6-159 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000587 | 2006/2/16 | 8.1 | |
| 第6回 | 6-161 | 感染症および寄生虫症 | A型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000589 | 2006/2/16 | 8.1 | |
| 第6回 | 6-032 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 14 | 1994 | 不明 | 症例報告 | 外国製品 | 05000458 | 2005/10/28 | 8.1 | |
| 第6回 | 4-06 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 11 | 不明 | 不明 | 症例報告 | 外国製品 | 04000081 | 2005/9/16 | 8.1 | 第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告 |
| 第6回 | 5-136 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000100 | 2005/10/27 | 8.0 | 第6回症例番号5-136は前回報告における第5回症例番号5-136において報告したものの追加報告 |
| 第6回 | 5-101 | 感染症および寄生虫症 | B型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000404 | 2005/10/21 | 8.1 | 第6回症例番号5-101は前回報告における第5回症例番号5-101において報告したものの追加報告 |
| 第6回 | 6-059 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000453 | 2005/10/25 | 8.1 | |
| 第6回 | 6-087 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000490 | 2005/12/20 | 8.1 | |
| 第6回 | 6-146 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000558 | 2006/2/13 | 8.1 | |
| 第6回 | 6-013 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000567 | 2006/2/13 | 8.1 | |
| 第6回 | 6-002 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 不明 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000569 | 2006/2/13 | 8.1 | |
| 第6回 | 6-003 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000585 | 2006/2/16 | 8.1 | |
| 第6回 | 6-163 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000591 | 2006/2/16 | 8.1 | |
| 第6回 | 6-166 | 感染症および寄生虫症 | B型肝炎 | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000598 | 2006/2/16 | 8.1 | |
| 第6回 | 6-176 | 感染症および寄生虫症 | B型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000613 | 2006/2/22 | 8.1 | |
| 第6回 | 6-007 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 1985 | 不明 | 症例報告 | 外国製品 | 05000571 | 2006/2/13 | 8.1 | |
| 第6回 | 6-015 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000619 | 2006/2/22 | 8.1 | |
| 第6回 | 6-025 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 05000536 | 2006/2/8 | 8.1 | |
| 第6回 | 6-026 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 05000537 | 2006/2/8 | 8.1 | |
| 第6回 | 6-032 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 14 | 1994 | 不明 | 症例報告 | 外国製品 | 05000458 | 2005/10/28 | 8.1 | |
| 第6回 | 6-035 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 32 | 1995/5/24 | 不明 | 症例報告 | 外国製品 | 05000607 | 2006/2/22 | 8.1 | |
| 第6回 | 6-037 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 2001 | 不明 | 症例報告 | 外国製品 | 05000562 | 2006/2/13 | 8.1 | |
| 第6回 | 6-038 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 女性 | 不明 | 2003 | 不明 | 症例報告 | 外国製品 | 05000628 | 2006/2/24 | 8.1 | |
| 第6回 | 4-06 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 11 | 不明 | 不明 | 症例報告 | 外国製品 | 04000081 | 2005/9/16 | 8.1 | 第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 感染症の種類 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 備考 識別番号 | 報告日 | 備考 MedDRA(Ver.) | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第6回 | 5-101 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000404 | 2005/10/21 | 8.1 | 第6回症例番号5-101は前回報告における第5回症例番号5-101において報告したものの追加報告 |
| 第6回 | 6-045 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000439 | 2005/9/9 | 8.0 | |
| 第6回 | 6-049 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000443 | 2005/9/14 | 8.0 | |
| 第6回 | 6-050 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 41 | 不明 | 不明 | 症例報告 | 外国製品 | 05000444 | 2005/9/14 | 8.0 | |
| 第6回 | 6-051 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000445 | 2005/9/14 | 8.0 | |
| 第6回 | 6-052 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000446 | 2005/9/14 | 8.0 | |
| 第6回 | 6-054 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000448 | 2005/9/16 | 8.0 | |
| 第6回 | 6-055 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 当該製品 | 05000449 | 2005/9/22 | 8.1 | |
| 第6回 | 6-056 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000450 | 2005/10/4 | 8.1 | |
| 第6回 | 6-057 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000451 | 2005/10/19 | 8.1 | |
| 第6回 | 6-058 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000452 | 2005/10/25 | 8.1 | |
| 第6回 | 6-059 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000453 | 2005/10/25 | 8.1 | |
| 第6回 | 6-060 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000454 | 2005/10/25 | 8.1 | |
| 第6回 | 6-061 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000455 | 2005/10/27 | 8.1 | |
| 第6回 | 6-062 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 24 | 不明 | 不明 | 症例報告 | 外国製品 | 05000457 | 2005/10/27 | 8.1 | |
| 第6回 | 6-063 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000459 | 2005/10/28 | 8.1 | |
| 第6回 | 6-064 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000460 | 2005/10/28 | 8.1 | |
| 第6回 | 6-066 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000464 | 2005/11/2 | 8.1 | |
| 第6回 | 6-069 | 感染症および寄生虫症 | C型肝炎 | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000467 | 2005/11/2 | 8.1 | |
| 第6回 | 6-070 | 感染症および寄生虫症 | C型肝炎 | ドミニカ共和国 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000468 | 2005/11/2 | 8.1 | |
| 第6回 | 6-071 | 感染症および寄生虫症 | C型肝炎 | ペルー | 不明 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000469 | 2005/11/2 | 8.1 | |
| 第6回 | 6-072 | 感染症および寄生虫症 | C型肝炎 | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000470 | 2005/11/2 | 8.1 | |
| 第6回 | 6-075 | 感染症および寄生虫症 | C型肝炎 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000478 | 2005/12/2 | 8.1 | |
| 第6回 | 6-076 | 感染症および寄生虫症 | C型肝炎 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000479 | 2005/12/2 | 8.1 | |
| 第6回 | 6-077 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000480 | 2005/12/2 | 8.1 | |
| 第6回 | 6-079 | 感染症および寄生虫症 | C型肝炎 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000482 | 2005/12/2 | 8.1 | |
| 第6回 | 6-080 | 感染症および寄生虫症 | C型肝炎 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000483 | 2005/12/2 | 8.1 | |
| 第6回 | 6-086 | 感染症および寄生虫症 | C型肝炎 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000489 | 2005/12/2 | 8.1 | |
| 第6回 | 6-087 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000490 | 2005/12/20 | 8.1 | |
| 第6回 | 6-088 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000496 | 2006/2/6 | 8.1 | |
| 第6回 | 6-125 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000533 | 2006/2/8 | 8.1 | |
| 第6回 | 6-126 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 未回復 | 症例報告 | 外国製品 | 05000534 | 2006/2/8 | 8.1 | |
| 第6回 | 6-128 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000538 | 2006/2/8 | 8.1 | |
| 第6回 | 6-129 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000539 | 2006/2/8 | 8.1 | |
| 第6回 | 6-131 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000543 | 2006/2/10 | 8.1 | |
| 第6回 | 6-132 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000544 | 2006/2/10 | 8.1 | |
| 第6回 | 6-133 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000545 | 2006/2/10 | 8.1 | |
| 第6回 | 6-134 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000546 | 2006/2/10 | 8.1 | |
| 第6回 | 6-135 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000547 | 2006/2/10 | 8.1 | |
| 第6回 | 6-136 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000548 | 2006/2/10 | 8.1 | |
| 第6回 | 6-144 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000556 | 2006/2/13 | 8.1 | |
| 第6回 | 6-145 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000557 | 2006/2/13 | 8.1 | |
| 第6回 | 6-146 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000558 | 2006/2/13 | 8.1 | |
| 第6回 | 6-147 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000573 | 2006/2/14 | 8.1 | |
| 第6回 | 6-148 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 未回復 | 症例報告 | 外国製品 | 05000559 | 2006/2/13 | 8.1 | |
| 第6回 | 6-149 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000560 | 2006/2/13 | 8.1 | |
| 第6回 | 6-150 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000561 | 2006/2/13 | 8.1 | |
| 第6回 | 6-152 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000564 | 2006/2/13 | 8.1 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第6回 | 6-153 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000565 | 2006/2/13 | 8.1 | |
| 第6回 | 6-154 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000566 | 2006/2/13 | 8.1 | |
| 第6回 | 6-013 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000567 | 2006/2/13 | 8.1 | |
| 第6回 | 6-155 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000568 | 2006/2/13 | 8.1 | |
| 第6回 | 6-157 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000579 | 2006/2/16 | 8.1 | |
| 第6回 | 6-158 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000580 | 2006/2/16 | 8.1 | |
| 第6回 | 6-001 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000582 | 2006/2/16 | 8.1 | |
| 第6回 | 6-159 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000587 | 2006/2/16 | 8.1 | |
| 第6回 | 6-160 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000588 | 2006/2/16 | 8.1 | |
| 第6回 | 6-161 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000589 | 2006/2/16 | 8.1 | |
| 第6回 | 6-162 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000590 | 2006/2/16 | 8.1 | |
| 第6回 | 6-163 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000591 | 2006/2/16 | 8.1 | |
| 第6回 | 6-014 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000592 | 2006/2/16 | 8.1 | |
| 第6回 | 6-018 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000593 | 2006/2/16 | 8.1 | |
| 第6回 | 6-019 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000594 | 2006/2/16 | 8.1 | |
| 第6回 | 6-004 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000595 | 2006/2/16 | 8.1 | |
| 第6回 | 6-164 | 感染症および寄生虫症 | C型肝炎 | パラグアイ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000596 | 2006/2/16 | 8.1 | |
| 第6回 | 6-165 | 感染症および寄生虫症 | C型肝炎 | パラグアイ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000597 | 2006/2/16 | 8.1 | |
| 第6回 | 6-166 | 感染症および寄生虫症 | C型肝炎 | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000598 | 2006/2/16 | 8.1 | |
| 第6回 | 6-169 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000601 | 2006/2/21 | 8.1 | |
| 第6回 | 6-173 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000605 | 2006/2/21 | 8.1 | |
| 第6回 | 6-174 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000606 | 2006/2/21 | 8.1 | |
| 第6回 | 6-022 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000609 | 2006/2/22 | 8.1 | |
| 第6回 | 6-175 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000612 | 2006/2/22 | 8.1 | |
| 第6回 | 6-024 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000624 | 2006/2/24 | 8.1 | |
| 第6回 | 6-183 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000625 | 2006/2/24 | 8.1 | |
| 第6回 | 6-184 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000626 | 2006/2/24 | 8.1 | |
| 第6回 | 6-185 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000627 | 2006/2/24 | 8.1 | |
| 第6回 | 6-012 | 感染症および寄生虫症 | C型肝炎 | 台湾 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000629 | 2006/2/24 | 8.1 | |
| 第6回 | 6-010 | 感染症および寄生虫症 | C型肝炎 | 台湾 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000630 | 2006/2/24 | 8.1 | |
| 第6回 | 6-011 | 感染症および寄生虫症 | C型肝炎 | 台湾 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000633 | 2006/2/24 | 8.1 | |
| 第6回 | 6-001 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1983 | 不明 | 症例報告 | 外国製品 | 05000582 | 2006/2/16 | 8.1 | |
| 第6回 | 6-002 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 不明 | 不明 | 1984 | 不明 | 症例報告 | 外国製品 | 05000569 | 2006/2/13 | 8.1 | |
| 第6回 | 6-003 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1984 | 不明 | 症例報告 | 外国製品 | 05000585 | 2006/2/16 | 8.1 | |
| 第6回 | 6-004 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1984 | 不明 | 症例報告 | 外国製品 | 05000595 | 2006/2/16 | 8.1 | |
| 第6回 | 6-005 | 感染症および寄生虫症 | HIV感染 | 台湾 | 男性 | 不明 | 1984/10/18 | 不明 | 症例報告 | 外国製品 | 05000620 | 2006/2/22 | 8.1 | |
| 第6回 | 6-006 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1985 | 不明 | 症例報告 | 外国製品 | 05000570 | 2006/2/13 | 8.1 | |
| 第6回 | 6-008 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1985 | 不明 | 症例報告 | 外国製品 | 05000608 | 2006/2/22 | 8.1 | |
| 第6回 | 6-009 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1985 | 不明 | 症例報告 | 外国製品 | 05000610 | 2006/2/22 | 8.1 | |
| 第6回 | 6-010 | 感染症および寄生虫症 | HIV感染 | 台湾 | 男性 | 不明 | 1985/1/3 | 不明 | 症例報告 | 外国製品 | 05000630 | 2006/2/24 | 8.1 | |
| 第6回 | 6-011 | 感染症および寄生虫症 | HIV感染 | 台湾 | 男性 | 不明 | 1985/11/13 | 不明 | 症例報告 | 外国製品 | 05000633 | 2006/2/24 | 8.1 | |
| 第6回 | 6-012 | 感染症および寄生虫症 | HIV感染 | 台湾 | 男性 | 不明 | 1985/5/1 | 不明 | 症例報告 | 外国製品 | 05000629 | 2006/2/24 | 8.1 | |
| 第6回 | 6-013 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000567 | 2006/2/13 | 8.1 | |
| 第6回 | 6-014 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000592 | 2006/2/16 | 8.1 | |
| 第6回 | 6-015 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000619 | 2006/2/22 | 8.1 | |
| 第6回 | 5-136 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000100 | 2005/10/27 | 8.0 | 第6回症例番号5-136は前回報告における第5回症例番号5-136において報告したものの追加報告 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | MedDRA (Ver.) | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第6回 | 5-101 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 1986/7/16 | 不明 | 症例報告 | 外国製品 | 05000404 | 2005/10/21 | 8.1 | 第6回症例番号5-101は前回報告における第5回症例番号5-101において報告したものの追加報告 |
| 第6回 | 6-016 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1987 | 不明 | 症例報告 | 外国製品 | 05000541 | 2006/2/9 | 8.1 | |
| 第6回 | 6-017 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 女性 | 不明 | 1987 | 不明 | 症例報告 | 外国製品 | 05000581 | 2006/2/16 | 8.1 | |
| 第6回 | 6-018 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1987 | 不明 | 症例報告 | 外国製品 | 05000593 | 2006/2/16 | 8.1 | |
| 第6回 | 6-019 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1987 | 不明 | 症例報告 | 外国製品 | 05000594 | 2006/2/16 | 8.1 | |
| 第6回 | 6-036 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 2000 | 不明 | 症例報告 | 外国製品 | 05000542 | 2006/2/9 | 8.1 | |
| 第6回 | 6-020 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 1988 | 不明 | 症例報告 | 外国製品 | 05000576 | 2006/2/16 | 8.1 | |
| 第6回 | 6-021 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1988 | 不明 | 症例報告 | 外国製品 | 05000584 | 2006/2/16 | 8.1 | |
| 第6回 | 6-022 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1989 | 不明 | 症例報告 | 外国製品 | 05000609 | 2006/2/22 | 8.1 | |
| 第6回 | 6-023 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1989 | 不明 | 症例報告 | 外国製品 | 05000611 | 2006/2/22 | 8.1 | |
| 第6回 | 6-024 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1989 | 不明 | 症例報告 | 外国製品 | 05000624 | 2006/2/24 | 8.1 | |
| 第6回 | 6-028 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 05000623 | 2006/2/24 | 8.1 | |
| 第6回 | 6-029 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 1990/1/3 | 不明 | 症例報告 | 外国製品 | 05000578 | 2006/2/16 | 8.1 | |
| 第6回 | 6-030 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1992 | 不明 | 症例報告 | 外国製品 | 05000583 | 2006/2/16 | 8.1 | |
| 第6回 | 6-034 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1994 | 不明 | 症例報告 | 外国製品 | 05000586 | 2006/2/16 | 8.1 | |
| 第6回 | 5-271 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000403 | 2005/10/27 | 8.0 | 第6回症例番号5-271は第6回症例番号5-101と重複症例のため報告破棄 |
| 第6回 | 6-040 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000434 | 2005/9/1 | 8.0 | |
| 第6回 | 6-045 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000439 | 2005/9/9 | 8.0 | |
| 第6回 | 6-046 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000440 | 2005/9/9 | 8.0 | |
| 第6回 | 6-048 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000442 | 2005/9/9 | 8.0 | |
| 第6回 | 6-053 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000447 | 2005/9/16 | 8.0 | |
| 第6回 | 6-065 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000463 | 2005/11/2 | 8.1 | |
| 第6回 | 6-066 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000464 | 2005/11/2 | 8.1 | |
| 第6回 | 6-067 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000465 | 2005/11/2 | 8.1 | |
| 第6回 | 6-068 | 感染症および寄生虫症 | HIV感染 | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000466 | 2005/11/2 | 8.1 | |
| 第6回 | 6-071 | 感染症および寄生虫症 | HIV感染 | ペルー | 不明 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000469 | 2005/11/2 | 8.1 | |
| 第6回 | 6-072 | 感染症および寄生虫症 | HIV感染 | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000470 | 2005/11/2 | 8.1 | |
| 第6回 | 6-076 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000479 | 2005/12/2 | 8.1 | |
| 第6回 | 6-077 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000480 | 2005/12/2 | 8.1 | |
| 第6回 | 6-078 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000481 | 2005/12/2 | 8.1 | |
| 第6回 | 6-080 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000483 | 2005/12/2 | 8.1 | |
| 第6回 | 6-081 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000484 | 2005/12/2 | 8.1 | |
| 第6回 | 6-082 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000485 | 2005/12/2 | 8.1 | |
| 第6回 | 6-083 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000486 | 2005/12/2 | 8.1 | |
| 第6回 | 6-084 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000487 | 2005/12/2 | 8.1 | |
| 第6回 | 6-085 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000488 | 2005/12/2 | 8.1 | |
| 第6回 | 6-086 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000489 | 2005/12/2 | 8.1 | |
| 第6回 | 6-090 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000498 | 2006/2/6 | 8.1 | |
| 第6回 | 6-101 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000509 | 2006/2/8 | 8.1 | |
| 第6回 | 6-105 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000513 | 2006/2/8 | 8.1 | |
| 第6回 | 6-107 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000515 | 2006/2/8 | 8.1 | |
| 第6回 | 6-108 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000516 | 2006/2/8 | 8.1 | |
| 第6回 | 6-111 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000519 | 2006/2/8 | 8.1 | |
| 第6回 | 6-112 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000520 | 2006/2/8 | 8.1 | |
| 第6回 | 6-117 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000525 | 2006/2/8 | 8.1 | |
| 第6回 | 6-118 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000526 | 2006/2/8 | 8.1 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第6回 | 6-144 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000556 | 2006/2/13 | 8.1 | |
| 第6回 | 6-162 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000590 | 2006/2/16 | 8.1 | |
| 第6回 | 6-176 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000613 | 2006/2/22 | 8.1 | |
| 第6回 | 4-06 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 11 | 1981/11/23 | 不明 | 症例報告 | 外国製品 | 04000081 | 2005/9/16 | 8.1 | 第6回症例番号4-06は前回報告における第4回症例番号4-06において報告したものの追加報告 |
| 第6回 | 6-020 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 不明 | 1988 | 不明 | 症例報告 | 外国製品 | 05000576 | 2006/2/16 | 8.1 | |
| 第6回 | 6-027 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 05000575 | 2006/2/16 | 8.1 | |
| 第6回 | 6-031 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 不明 | 1993 | 不明 | 症例報告 | 外国製品 | 05000618 | 2006/2/22 | 8.1 | |
| 第6回 | 5-286 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 13 | 1994 | 不明 | 症例報告 | 外国製品 | 05000273 | 2006/2/15 | 8.0 | 第6回症例番号5-286は第6回症例番号6-033と重複症例のため報告破棄 |
| 第6回 | 6-033 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 13 | 1994 | 不明 | 症例報告 | 外国製品 | 05000572 | 2006/2/13 | 8.1 | |
| 第6回 | 6-036 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 不明 | 2000 | 不明 | 症例報告 | 外国製品 | 05000542 | 2006/2/9 | 8.1 | |
| 第6回 | 6-151 | 肝胆道系障害 | 肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000563 | 2006/2/13 | 8.1 | |
| 第6回 | 6-156 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000577 | 2006/2/16 | 8.1 | |
| 第6回 | 6-089 | 社会環境 | 伝染病暴露 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000497 | 2006/2/6 | 8.1 | |
| 第6回 | 6-093 | 社会環境 | 伝染病暴露 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000501 | 2006/2/6 | 8.1 | |
| 第6回 | 6-096 | 社会環境 | 伝染病暴露 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000503 | 2006/2/6 | 8.1 | |
| 第6回 | 6-097 | 社会環境 | 伝染病暴露 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000504 | 2006/2/6 | 8.1 | |
| 第6回 | 6-091 | 臨床検査 | A型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000499 | 2006/2/6 | 8.1 | |
| 第6回 | 6-137 | 臨床検査 | A型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000549 | 2006/2/10 | 8.1 | |
| 第6回 | 6-091 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000499 | 2006/2/6 | 8.1 | |
| 第6回 | 6-137 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000549 | 2006/2/10 | 8.1 | |
| 第6回 | 6-142 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000554 | 2006/2/10 | 8.1 | |
| 第6回 | 6-187 | 臨床検査 | B型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000632 | 2006/2/24 | 8.1 | |
| 第6回 | 5-136 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000100 | 2005/10/27 | 8.0 | 第6回症例番号5-136は前回報告における第5回症例番号5-136において報告したものの追加報告 |
| 第6回 | 5-271 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000403 | 2005/10/27 | 8.0 | 第6回症例番号5-271は第6回症例番号5-101と重複症例のため報告破棄 |
| 第6回 | 6-039 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000433 | 2005/9/1 | 8.0 | |
| 第6回 | 6-040 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000434 | 2005/9/1 | 8.0 | |
| 第6回 | 6-041 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000435 | 2005/9/1 | 8.0 | |
| 第6回 | 6-042 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000436 | 2005/9/1 | 8.0 | |
| 第6回 | 6-043 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000437 | 2005/9/1 | 8.0 | |
| 第6回 | 6-044 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000438 | 2005/9/9 | 8.0 | |
| 第6回 | 6-046 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000440 | 2005/9/9 | 8.0 | |
| 第6回 | 6-047 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000441 | 2005/9/9 | 8.0 | |
| 第6回 | 6-065 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000463 | 2005/11/2 | 8.1 | |
| 第6回 | 6-067 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000465 | 2005/11/2 | 8.1 | |
| 第6回 | 6-068 | 臨床検査 | C型肝炎ウイルス | ペルー | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000466 | 2005/11/2 | 8.1 | |
| 第6回 | 6-073 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000473 | 2005/11/21 | 8.1 | |
| 第6回 | 6-074 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000476 | 2005/11/30 | 8.1 | |
| 第6回 | 6-090 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000498 | 2006/2/6 | 8.1 | |
| 第6回 | 6-091 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000499 | 2006/2/6 | 8.1 | |
| 第6回 | 6-092 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000500 | 2006/2/6 | 8.1 | |
| 第6回 | 6-094 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000502 | 2006/2/6 | 8.1 | |
| 第6回 | 6-095 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000506 | 2006/2/6 | 8.1 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第6回 | 6-098 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000505 | 2006/2/6 | 8.1 | |
| 第6回 | 6-099 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000507 | 2006/2/8 | 8.1 | |
| 第6回 | 6-100 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000508 | 2006/2/8 | 8.1 | |
| 第6回 | 6-101 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000509 | 2006/2/8 | 8.1 | |
| 第6回 | 6-102 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000510 | 2006/2/8 | 8.1 | |
| 第6回 | 6-103 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000511 | 2006/2/8 | 8.1 | |
| 第6回 | 6-104 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000512 | 2006/2/8 | 8.1 | |
| 第6回 | 6-105 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000513 | 2006/2/8 | 8.1 | |
| 第6回 | 6-106 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000514 | 2006/2/8 | 8.1 | |
| 第6回 | 6-109 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000517 | 2006/2/8 | 8.1 | |
| 第6回 | 6-110 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000518 | 2006/2/8 | 8.1 | |
| 第6回 | 6-113 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000521 | 2006/2/8 | 8.1 | |
| 第6回 | 6-114 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000522 | 2006/2/8 | 8.1 | |
| 第6回 | 6-115 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000523 | 2006/2/8 | 8.1 | |
| 第6回 | 6-116 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000524 | 2006/2/8 | 8.1 | |
| 第6回 | 6-118 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000526 | 2006/2/8 | 8.1 | |
| 第6回 | 6-119 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000527 | 2006/2/8 | 8.1 | |
| 第6回 | 6-120 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000528 | 2006/2/8 | 8.1 | |
| 第6回 | 6-121 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000529 | 2006/2/8 | 8.1 | |
| 第6回 | 6-122 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000530 | 2006/2/8 | 8.1 | |
| 第6回 | 6-123 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000531 | 2006/2/8 | 8.1 | |
| 第6回 | 6-124 | 臨床検査 | C型肝炎ウイルス | アメリカ | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000532 | 2006/2/8 | 8.1 | |
| 第6回 | 6-127 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000535 | 2006/2/8 | 8.1 | |
| 第6回 | 6-130 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000540 | 2006/2/9 | 8.1 | |
| 第6回 | 6-137 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000549 | 2006/2/10 | 8.1 | |
| 第6回 | 6-138 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000550 | 2006/2/10 | 8.1 | |
| 第6回 | 6-139 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000551 | 2006/2/10 | 8.1 | |
| 第6回 | 6-140 | 臨床検査 | C型肝炎ウイルス | アメリカ | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000552 | 2006/2/10 | 8.1 | |
| 第6回 | 6-141 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000553 | 2006/2/10 | 8.1 | |
| 第6回 | 6-142 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000554 | 2006/2/10 | 8.1 | |
| 第6回 | 6-143 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000555 | 2006/2/10 | 8.1 | |
| 第6回 | 6-029 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000578 | 2006/2/16 | 8.1 | |
| 第6回 | 6-167 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000599 | 2006/2/16 | 8.1 | |
| 第6回 | 6-168 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000600 | 2006/2/21 | 8.1 | |
| 第6回 | 6-170 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000602 | 2006/2/21 | 8.1 | |
| 第6回 | 6-171 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000603 | 2006/2/21 | 8.1 | |
| 第6回 | 6-172 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000604 | 2006/2/21 | 8.1 | |
| 第6回 | 6-177 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000614 | 2006/2/22 | 8.1 | |
| 第6回 | 6-178 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000615 | 2006/2/22 | 8.1 | |
| 第6回 | 6-179 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000616 | 2006/2/22 | 8.1 | |
| 第6回 | 6-180 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000617 | 2006/2/22 | 8.1 | |
| 第6回 | 6-181 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000621 | 2006/2/22 | 8.1 | |
| 第6回 | 6-182 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000622 | 2006/2/22 | 8.1 | |
| 第6回 | 6-186 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000631 | 2006/2/24 | 8.1 | |
| 第6回 | 6-187 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000632 | 2006/2/24 | 8.1 | |
| 第5回 | 5-001 | 感染症および寄生虫症 | A型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000118 | 2005/3/18 | 8.0 | |
| 第5回 | 5-002 | 感染症および寄生虫症 | A型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000244 | 2005/7/15 | 8.0 | |
| 第5回 | 5-003 | 感染症および寄生虫症 | A型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000245 | 2005/7/15 | 8.0 | |
| 第5回 | 5-004 | 感染症および寄生虫症 | B型肝炎 | ベネズエラ | 男性 | 不明 | 1994 | 不明 | 症例報告 | 外国製品 | 05000225 | 2005/7/11 | 8.0 | |
| 第5回 | 5-001 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000118 | 2005/3/18 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器直別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-002 | 感染症および寄生虫症 | B型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000244 | 2005/7/15 | 8.0 | |
| 第5回 | 5-003 | 感染症および寄生虫症 | B型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000245 | 2005/7/15 | 8.0 | |
| 第5回 | 5-005 | 感染症および寄生虫症 | B型肝炎 | アメリカ | 男性 | 53 | 不明 | 不明 | 症例報告 | 当該製品 | 04000114 | 2005/3/15 | 7.1 | |
| 第5回 | 5-006 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000119 | 2005/3/18 | 8.0 | |
| 第5回 | 5-007 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000118 | 2005/6/9 | 8.0 | |
| 第5回 | 5-008 | 感染症および寄生虫症 | B型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000147 | 2005/6/20 | 8.0 | |
| 第5回 | 5-004 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 1994 | 不明 | 症例報告 | 外国製品 | 05000225 | 2005/7/11 | 8.0 | |
| 第5回 | 5-009 | 感染症および寄生虫症 | C型肝炎 | イタリア | 男性 | 不明 | 1992 | 不明 | 症例報告 | 外国製品 | 04000127 | 2005/3/31 | 8.0 | |
| 第5回 | 5-001 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000118 | 2005/3/18 | 8.0 | |
| 第5回 | 5-002 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000244 | 2005/7/15 | 8.0 | |
| 第5回 | 5-003 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000245 | 2005/7/15 | 8.0 | |
| 第5回 | 5-005 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 53 | 不明 | 不明 | 症例報告 | 当該製品 | 04000114 | 2005/3/15 | 7.1 | |
| 第5回 | 5-006 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000119 | 2005/3/18 | 8.0 | |
| 第5回 | 5-007 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000118 | 2005/6/9 | 8.0 | |
| 第5回 | 5-008 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000147 | 2005/6/20 | 8.0 | |
| 第5回 | 5-010 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 52 | 不明 | 不明 | 症例報告 | 外国製品 | 04000103 | 2005/3/3 | 7.1 | |
| 第5回 | 5-011 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 21 | 不明 | 不明 | 症例報告 | 外国製品 | 04000106 | 2005/3/3 | 7.1 | |
| 第5回 | 5-012 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 49 | 不明 | 不明 | 症例報告 | 外国製品 | 04000111 | 2005/3/10 | 7.1 | |
| 第5回 | 5-013 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 24 | 不明 | 不明 | 症例報告 | 当該製品 | 04000112 | 2005/3/15 | 7.1 | |
| 第5回 | 5-014 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 35 | 不明 | 不明 | 症例報告 | 当該製品 | 04000113 | 2005/3/15 | 7.1 | |
| 第5回 | 5-015 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 26 | 不明 | 不明 | 症例報告 | 当該製品 | 04000115 | 2005/3/15 | 7.1 | |
| 第5回 | 5-016 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000117 | 2005/3/17 | 8.0 | |
| 第5回 | 5-017 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000005 | 2005/4/25 | 8.0 | |
| 第5回 | 5-018 | 感染症および寄生虫症 | C型肝炎 | スペイン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000007 | 2005/4/25 | 8.0 | |
| 第5回 | 5-019 | 感染症および寄生虫症 | C型肝炎 | スペイン | 男性 | 48 | 不明 | 不明 | 症例報告 | 外国製品 | 05000091 | 2005/6/1 | 8.0 | |
| 第5回 | 5-020 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000069 | 2005/6/1 | 8.0 | |
| 第5回 | 5-020 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000069 | 2005/6/15 | 8.0 | |
| 第5回 | 5-021 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000070 | 2005/6/1 | 8.0 | |
| 第5回 | 5-021 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000070 | 2005/6/15 | 8.0 | |
| 第5回 | 5-022 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000071 | 2005/6/1 | 8.0 | |
| 第5回 | 5-022 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000071 | 2005/6/15 | 8.0 | |
| 第5回 | 5-023 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000072 | 2005/6/1 | 8.0 | |
| 第5回 | 5-023 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000072 | 2005/6/15 | 8.0 | |
| 第5回 | 5-024 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000073 | 2005/6/1 | 8.0 | |
| 第5回 | 5-024 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000073 | 2005/6/15 | 8.0 | |
| 第5回 | 5-025 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000074 | 2005/6/1 | 8.0 | |
| 第5回 | 5-025 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000074 | 2005/6/15 | 8.0 | |
| 第5回 | 5-026 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000075 | 2005/6/1 | 8.0 | |
| 第5回 | 5-027 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000076 | 2005/6/1 | 8.0 | |
| 第5回 | 5-028 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000092 | 2005/6/1 | 8.0 | |
| 第5回 | 5-029 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000093 | 2005/6/1 | 8.0 | |
| 第5回 | 5-030 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000094 | 2005/6/1 | 8.0 | |
| 第5回 | 5-031 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000095 | 2005/6/1 | 8.0 | |
| 第5回 | 5-032 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000096 | 2005/6/1 | 8.0 | |
| 第5回 | 5-033 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000097 | 2005/6/1 | 8.0 | |
| 第5回 | 5-034 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000077 | 2005/6/1 | 8.0 | |
| 第5回 | 5-035 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000078 | 2005/6/1 | 8.0 | |
| 第5回 | 5-035 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000078 | 2005/6/15 | 8.0 | |
| 第5回 | 5-036 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000079 | 2005/6/1 | 8.0 | |
| 第5回 | 5-037 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000080 | 2005/6/1 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA(Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 部位別大分類 | 基本語(PT) | | | | | | | | | | |
| 第5回 | 5-038 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000081 | 2005/6/1 | 8.0 |
| 第5回 | 5-039 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000082 | 2005/6/1 | 8.0 |
| 第5回 | 5-040 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000083 | 2005/6/1 | 8.0 |
| 第5回 | 5-041 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000084 | 2005/6/1 | 8.0 |
| 第5回 | 5-042 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000088 | 2005/6/1 | 8.0 |
| 第5回 | 5-043 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000089 | 2005/6/1 | 8.0 |
| 第5回 | 5-043 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000089 | 2005/6/15 | 8.0 |
| 第5回 | 5-044 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000090 | 2005/6/1 | 8.0 |
| 第5回 | 5-044 | 感染症および寄生虫症 | C型肝炎 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000090 | 2005/6/15 | 8.0 |
| 第5回 | 5-045 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000109 | 2005/6/9 | 8.0 |
| 第5回 | 5-046 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000114 | 2005/6/9 | 8.0 |
| 第5回 | 5-047 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000135 | 2005/6/20 | 8.0 |
| 第5回 | 5-048 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000136 | 2005/6/20 | 8.0 |
| 第5回 | 5-049 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000137 | 2005/6/20 | 8.0 |
| 第5回 | 5-050 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000144 | 2005/6/20 | 8.0 |
| 第5回 | 5-051 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000145 | 2005/6/20 | 8.0 |
| 第5回 | 5-052 | 感染症および寄生虫症 | C型肝炎 | コロンビア | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000150 | 2005/6/27 | 8.0 |
| 第5回 | 5-053 | 感染症および寄生虫症 | C型肝炎 | ドミニカ共和国 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000155 | 2005/6/27 | 8.0 |
| 第5回 | 5-054 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000183 | 2005/7/4 | 8.0 |
| 第5回 | 5-055 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000195 | 2005/7/8 | 8.0 |
| 第5回 | 5-056 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000196 | 2005/7/8 | 8.0 |
| 第5回 | 5-057 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000215 | 2005/7/11 | 8.0 |
| 第5回 | 5-058 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000218 | 2005/7/11 | 8.0 |
| 第5回 | 5-059 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000221 | 2005/7/11 | 8.0 |
| 第5回 | 5-060 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000223 | 2005/7/11 | 8.0 |
| 第5回 | 5-061 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000226 | 2005/7/11 | 8.0 |
| 第5回 | 5-062 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000227 | 2005/7/11 | 8.0 |
| 第5回 | 5-063 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000228 | 2005/7/11 | 8.0 |
| 第5回 | 5-064 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000231 | 2005/7/12 | 8.0 |
| 第5回 | 5-065 | 感染症および寄生虫症 | C型肝炎 | ドミニカ共和国 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000234 | 2005/7/12 | 8.0 |
| 第5回 | 5-066 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000238 | 2005/7/15 | 8.0 |
| 第5回 | 5-067 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000239 | 2005/7/15 | 8.0 |
| 第5回 | 5-068 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000243 | 2005/7/15 | 8.0 |
| 第5回 | 5-069 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000246 | 2005/7/15 | 8.0 |
| 第5回 | 5-070 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000253 | 2005/7/19 | 8.0 |
| 第5回 | 5-070 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000253 | 2005/8/2 | 8.0 |
| 第5回 | 5-071 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000262 | 2005/7/22 | 8.0 |
| 第5回 | 5-072 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000263 | 2005/7/22 | 8.0 |
| 第5回 | 5-073 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000281 | 2005/7/26 | 8.0 |
| 第5回 | 5-074 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000310 | 2005/7/27 | 8.0 |
| 第5回 | 5-075 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000311 | 2005/7/27 | 8.0 |
| 第5回 | 5-076 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000312 | 2005/7/27 | 8.0 |
| 第5回 | 5-077 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000324 | 2005/7/27 | 8.0 |
| 第5回 | 5-078 | 感染症および寄生虫症 | C型肝炎 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000325 | 2005/7/27 | 8.0 |
| 第5回 | 5-079 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000327 | 2005/7/29 | 8.0 |
| 第5回 | 5-080 | 感染症および寄生虫症 | C型肝炎 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000328 | 2005/7/29 | 8.0 |
| 第5回 | 5-081 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000347 | 2005/8/2 | 8.0 |
| 第5回 | 5-082 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000348 | 2005/8/2 | 8.0 |
| 第5回 | 5-083 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000349 | 2005/8/2 | 8.0 |
| 第5回 | 5-084 | 感染症および寄生虫症 | C型肝炎 | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000352 | 2005/8/2 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 感染症の種類 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 備考 識別番号 | 備考 報告日 | 備考 MedDRA(Ver.) | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-085 | 感染症および寄生虫症 | C型肝炎 | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000361 | 2005/8/2 | 8.0 | |
| 第5回 | 5-086 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000363 | 2005/8/2 | 8.0 | |
| 第5回 | 5-087 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000364 | 2005/8/2 | 8.0 | |
| 第5回 | 5-088 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000365 | 2005/8/2 | 8.0 | |
| 第5回 | 5-089 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000366 | 2005/8/2 | 8.0 | |
| 第5回 | 5-090 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000367 | 2005/8/3 | 8.0 | |
| 第5回 | 5-091 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000369 | 2005/8/3 | 8.0 | |
| 第5回 | 5-092 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000371 | 2005/8/3 | 8.0 | |
| 第5回 | 5-093 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000372 | 2005/8/3 | 8.0 | |
| 第5回 | 5-094 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000375 | 2005/8/3 | 8.0 | |
| 第5回 | 5-095 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000376 | 2005/8/3 | 8.0 | |
| 第5回 | 5-096 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000379 | 2005/8/3 | 8.0 | |
| 第5回 | 5-097 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000380 | 2005/8/3 | 8.0 | |
| 第5回 | 5-098 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000386 | 2005/8/3 | 8.0 | |
| 第5回 | 5-099 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000392 | 2005/8/8 | 8.0 | |
| 第5回 | 5-100 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000393 | 2005/8/8 | 8.0 | |
| 第5回 | 5-101 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000404 | 2005/8/15 | 8.0 | |
| 第5回 | 5-102 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000416 | 2005/8/26 | 8.0 | |
| 第5回 | 5-103 | 感染症および寄生虫症 | C型肝炎 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000419 | 2005/8/26 | 8.0 | |
| 第5回 | 5-009 | 感染症および寄生虫症 | HIV感染 | イタリア | 男性 | 不明 | 1985/3 | 不明 | 症例報告 | 外国製品 | 04000127 | 2005/3/31 | 8.0 | |
| 第5回 | 5-072 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 1986 | 不明 | 症例報告 | 外国製品 | 05000263 | 2005/7/22 | 8.0 | |
| 第5回 | 5-080 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 05000328 | 2005/7/29 | 8.0 | |
| 第5回 | 5-002 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000244 | 2005/7/15 | 8.0 | |
| 第5回 | 5-003 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000245 | 2005/7/15 | 8.0 | |
| 第5回 | 5-017 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000005 | 2005/4/25 | 8.0 | |
| 第5回 | 5-018 | 感染症および寄生虫症 | HIV感染 | スペイン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000007 | 2005/4/25 | 8.0 | |
| 第5回 | 5-020 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000069 | 2005/6/1 | 8.0 | |
| 第5回 | 5-020 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000069 | 2005/6/15 | 8.0 | |
| 第5回 | 5-021 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000070 | 2005/6/1 | 8.0 | |
| 第5回 | 5-021 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000070 | 2005/6/15 | 8.0 | |
| 第5回 | 5-022 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000071 | 2005/6/1 | 8.0 | |
| 第5回 | 5-022 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000071 | 2005/6/15 | 8.0 | |
| 第5回 | 5-023 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000072 | 2005/6/1 | 8.0 | |
| 第5回 | 5-023 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000072 | 2005/6/15 | 8.0 | |
| 第5回 | 5-024 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000073 | 2005/6/1 | 8.0 | |
| 第5回 | 5-024 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000073 | 2005/6/15 | 8.0 | |
| 第5回 | 5-025 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000074 | 2005/6/15 | 8.0 | |
| 第5回 | 5-025 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000074 | 2005/6/1 | 8.0 | |
| 第5回 | 5-026 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000075 | 2005/6/1 | 8.0 | |
| 第5回 | 5-027 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000076 | 2005/6/1 | 8.0 | |
| 第5回 | 5-028 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000092 | 2005/6/1 | 8.0 | |
| 第5回 | 5-029 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000093 | 2005/6/1 | 8.0 | |
| 第5回 | 5-030 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000094 | 2005/6/1 | 8.0 | |
| 第5回 | 5-031 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000095 | 2005/6/1 | 8.0 | |
| 第5回 | 5-032 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000096 | 2005/6/1 | 8.0 | |
| 第5回 | 5-033 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000097 | 2005/6/1 | 8.0 | |
| 第5回 | 5-034 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000077 | 2005/6/1 | 8.0 | |
| 第5回 | 5-035 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000078 | 2005/6/1 | 8.0 | |
| 第5回 | 5-035 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000078 | 2005/6/15 | 8.0 | |
| 第5回 | 5-036 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000079 | 2005/6/1 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 感染症別大分類 | | | | | | | | | | | | | |
| 第5回 | 5-037 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000080 | 2005/6/1 | 8.0 | |
| 第5回 | 5-038 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000081 | 2005/6/1 | 8.0 | |
| 第5回 | 5-039 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000082 | 2005/6/1 | 8.0 | |
| 第5回 | 5-040 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000083 | 2005/6/1 | 8.0 | |
| 第5回 | 5-041 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000084 | 2005/6/1 | 8.0 | |
| 第5回 | 5-042 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000088 | 2005/6/1 | 8.0 | |
| 第5回 | 5-053 | 感染症および寄生虫症 | | HIV感染 | ドミニカ共和国 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000155 | 2005/6/27 | 8.0 | |
| 第5回 | 5-055 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000195 | 2005/7/8 | 8.0 | |
| 第5回 | 5-056 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000196 | 2005/7/8 | 8.0 | |
| 第5回 | 5-067 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000239 | 2005/7/15 | 8.0 | |
| 第5回 | 5-068 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000243 | 2005/7/15 | 8.0 | |
| 第5回 | 5-069 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000246 | 2005/7/15 | 8.0 | |
| 第5回 | 5-071 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000262 | 2005/7/22 | 8.0 | |
| 第5回 | 5-073 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000281 | 2005/7/26 | 8.0 | |
| 第5回 | 5-079 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000327 | 2005/7/29 | 8.0 | |
| 第5回 | 5-082 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000348 | 2005/8/2 | 8.0 | |
| 第5回 | 5-083 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000349 | 2005/8/2 | 8.0 | |
| 第5回 | 5-085 | 感染症および寄生虫症 | | HIV感染 | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000361 | 2005/8/2 | 8.0 | |
| 第5回 | 5-089 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000366 | 2005/8/2 | 8.0 | |
| 第5回 | 5-096 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000379 | 2005/8/3 | 8.0 | |
| 第5回 | 5-097 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000380 | 2005/8/3 | 8.0 | |
| 第5回 | 5-100 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000393 | 2005/8/8 | 8.0 | |
| 第5回 | 5-101 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000404 | 2005/8/15 | 8.0 | |
| 第5回 | 5-103 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000419 | 2005/8/26 | 8.0 | |
| 第5回 | 5-104 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000004 | 2005/4/25 | 8.0 | |
| 第5回 | 5-105 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000006 | 2005/4/25 | 8.0 | |
| 第5回 | 5-106 | 感染症および寄生虫症 | | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000008 | 2005/4/28 | 8.0 | |
| 第5回 | 5-107 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000012 | 2005/5/12 | 8.0 | |
| 第5回 | 5-108 | 感染症および寄生虫症 | | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000013 | 2005/5/12 | 8.0 | |
| 第5回 | 5-109 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000015 | 2005/5/23 | 8.0 | |
| 第5回 | 5-110 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000016 | 2005/5/23 | 8.0 | |
| 第5回 | 5-111 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000017 | 2005/5/23 | 8.0 | |
| 第5回 | 5-112 | 感染症および寄生虫症 | | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000018 | 2005/5/23 | 8.0 | |
| 第5回 | 5-113 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000019 | 2005/5/23 | 8.0 | |
| 第5回 | 5-114 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000020 | 2005/5/23 | 8.0 | |
| 第5回 | 5-115 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000021 | 2005/5/23 | 8.0 | |
| 第5回 | 5-116 | 感染症および寄生虫症 | | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000022 | 2005/5/23 | 8.0 | |
| 第5回 | 5-117 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000023 | 2005/5/23 | 8.0 | |
| 第5回 | 5-118 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000025 | 2005/5/26 | 8.0 | |
| 第5回 | 5-119 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000026 | 2005/5/26 | 8.0 | |
| 第5回 | 5-120 | 感染症および寄生虫症 | | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000027 | 2005/5/26 | 8.0 | |
| 第5回 | 5-121 | 感染症および寄生虫症 | | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000053 | 2005/5/30 | 8.0 | |
| 第5回 | 5-122 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000054 | 2005/5/30 | 8.0 | |
| 第5回 | 5-123 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000056 | 2005/5/30 | 8.0 | |
| 第5回 | 5-124 | 感染症および寄生虫症 | | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000057 | 2005/5/30 | 8.0 | |
| 第5回 | 5-125 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000058 | 2005/5/30 | 8.0 | |
| 第5回 | 5-126 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000061 | 2005/5/30 | 8.0 | |
| 第5回 | 5-127 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000062 | 2005/5/30 | 8.0 | |
| 第5回 | 5-128 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000063 | 2005/5/30 | 8.0 | |
| 第5回 | 5-129 | 感染症および寄生虫症 | | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000064 | 2005/5/30 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA(Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語(PT) | | | | | | | | | | |
| 第5回 | 5-130 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000065 | 2005/5/30 | 8.0 |
| 第5回 | 5-131 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000066 | 2005/5/30 | 8.0 |
| 第5回 | 5-132 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000067 | 2005/5/30 | 8.0 |
| 第5回 | 5-133 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000068 | 2005/5/30 | 8.0 |
| 第5回 | 5-134 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000098 | 2005/6/1 | 8.0 |
| 第5回 | 5-135 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000099 | 2005/6/1 | 8.0 |
| 第5回 | 5-136 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000100 | 2005/6/1 | 8.0 |
| 第5回 | 5-137 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000102 | 2005/6/1 | 8.0 |
| 第5回 | 5-138 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000103 | 2005/6/2 | 8.0 |
| 第5回 | 5-139 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000104 | 2005/6/2 | 8.0 |
| 第5回 | 5-140 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000105 | 2005/6/2 | 8.0 |
| 第5回 | 5-141 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000085 | 2005/6/1 | 8.0 |
| 第5回 | 5-142 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000086 | 2005/6/1 | 8.0 |
| 第5回 | 5-142 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000086 | 2005/6/15 | 8.0 |
| 第5回 | 5-143 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000087 | 2005/6/1 | 8.0 |
| 第5回 | 5-143 | 感染症および寄生虫症 | HIV感染 | 南アフリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000087 | 2005/6/15 | 8.0 |
| 第5回 | 5-144 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000030 | 2005/5/30 | 8.0 |
| 第5回 | 5-144 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000030 | 2005/6/15 | 8.0 |
| 第5回 | 5-145 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000031 | 2005/5/30 | 8.0 |
| 第5回 | 5-145 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000031 | 2005/6/15 | 8.0 |
| 第5回 | 5-146 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000032 | 2005/5/30 | 8.0 |
| 第5回 | 5-146 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000032 | 2005/6/15 | 8.0 |
| 第5回 | 5-147 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000033 | 2005/5/30 | 8.0 |
| 第5回 | 5-148 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000034 | 2005/5/30 | 8.0 |
| 第5回 | 5-149 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000035 | 2005/5/30 | 8.0 |
| 第5回 | 5-150 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000036 | 2005/5/30 | 8.0 |
| 第5回 | 5-151 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000111 | 2005/6/9 | 8.0 |
| 第5回 | 5-152 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000112 | 2005/6/9 | 8.0 |
| 第5回 | 5-153 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 4 | 不明 | 不明 | 症例報告 | 外国製品 | 05000113 | 2005/6/9 | 8.0 |
| 第5回 | 5-154 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000037 | 2005/5/30 | 8.0 |
| 第5回 | 5-155 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000038 | 2005/5/30 | 8.0 |
| 第5回 | 5-156 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000039 | 2005/5/30 | 8.0 |
| 第5回 | 5-157 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000040 | 2005/5/30 | 8.0 |
| 第5回 | 5-158 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000116 | 2005/6/9 | 8.0 |
| 第5回 | 5-159 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000041 | 2005/5/30 | 8.0 |
| 第5回 | 5-160 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000042 | 2005/5/30 | 8.0 |
| 第5回 | 5-161 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000043 | 2005/5/30 | 8.0 |
| 第5回 | 5-162 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000044 | 2005/5/30 | 8.0 |
| 第5回 | 5-162 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000044 | 2005/6/27 | 8.0 |
| 第5回 | 5-163 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000045 | 2005/5/30 | 8.0 |
| 第5回 | 5-163 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000045 | 2005/6/15 | 8.0 |
| 第5回 | 5-164 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000046 | 2005/5/30 | 8.0 |
| 第5回 | 5-165 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000047 | 2005/5/30 | 8.0 |
| 第5回 | 5-166 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000048 | 2005/5/30 | 8.0 |
| 第5回 | 5-167 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000049 | 2005/5/30 | 8.0 |
| 第5回 | 5-167 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000049 | 2005/6/15 | 8.0 |
| 第5回 | 5-168 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000050 | 2005/5/30 | 8.0 |
| 第5回 | 5-168 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000050 | 2005/6/15 | 8.0 |
| 第5回 | 5-169 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000051 | 2005/5/30 | 8.0 |
| 第5回 | 5-169 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000051 | 2005/6/15 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 報告別大分類 | 基本語 (PT) | 発現国 | 性別 | 年齢 (歳) | 発現時期 (年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 受報告日 | 備考 MedDRA (Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-170 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000052 | 2005/5/30 | 8.0 |
| 第5回 | 5-170 | 感染症および寄生虫症 | HIV感染 | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000052 | 2005/6/15 | 8.0 |
| 第5回 | 5-171 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000119 | 2005/6/13 | 8.0 |
| 第5回 | 5-172 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000120 | 2005/6/13 | 8.0 |
| 第5回 | 5-173 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000121 | 2005/6/13 | 8.0 |
| 第5回 | 5-174 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000122 | 2005/6/13 | 8.0 |
| 第5回 | 5-175 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000124 | 2005/6/13 | 8.0 |
| 第5回 | 5-176 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000126 | 2005/6/15 | 8.0 |
| 第5回 | 5-177 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000129 | 2005/6/15 | 8.0 |
| 第5回 | 5-178 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000133 | 2005/6/20 | 8.0 |
| 第5回 | 5-179 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000134 | 2005/6/20 | 8.0 |
| 第5回 | 5-180 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000148 | 2005/6/20 | 8.0 |
| 第5回 | 5-181 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000156 | 2005/6/27 | 8.0 |
| 第5回 | 5-182 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000159 | 2005/6/27 | 8.0 |
| 第5回 | 5-183 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000161 | 2005/6/27 | 8.0 |
| 第5回 | 5-184 | 感染症および寄生虫症 | HIV感染 | チリ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000162 | 2005/6/27 | 8.0 |
| 第5回 | 5-185 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000164 | 2005/6/27 | 8.0 |
| 第5回 | 5-186 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000165 | 2005/6/27 | 8.0 |
| 第5回 | 5-187 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000167 | 2005/6/27 | 8.0 |
| 第5回 | 5-188 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000171 | 2005/6/30 | 8.0 |
| 第5回 | 5-189 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000173 | 2005/6/30 | 8.0 |
| 第5回 | 5-190 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000175 | 2005/6/30 | 8.0 |
| 第5回 | 5-191 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000176 | 2005/6/30 | 8.0 |
| 第5回 | 5-192 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000181 | 2005/6/30 | 8.0 |
| 第5回 | 5-193 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000182 | 2005/6/30 | 8.0 |
| 第5回 | 5-193 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000182 | 2005/8/8 | 8.0 |
| 第5回 | 5-194 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000185 | 2005/7/4 | 8.0 |
| 第5回 | 5-195 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000186 | 2005/7/4 | 8.0 |
| 第5回 | 5-196 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000187 | 2005/7/4 | 8.0 |
| 第5回 | 5-197 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000189 | 2005/7/4 | 8.0 |
| 第5回 | 5-198 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000190 | 2005/7/4 | 8.0 |
| 第5回 | 5-199 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000191 | 2005/7/4 | 8.0 |
| 第5回 | 5-200 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000194 | 2005/7/4 | 8.0 |
| 第5回 | 5-201 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000202 | 2005/7/8 | 8.0 |
| 第5回 | 5-202 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000203 | 2005/7/8 | 8.0 |
| 第5回 | 5-203 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000204 | 2005/7/8 | 8.0 |
| 第5回 | 5-204 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000206 | 2005/7/8 | 8.0 |
| 第5回 | 5-205 | 感染症および寄生虫症 | HIV感染 | コロンビア | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000209 | 2005/7/8 | 8.0 |
| 第5回 | 5-206 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000212 | 2005/7/8 | 8.0 |
| 第5回 | 5-207 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000213 | 2005/7/11 | 8.0 |
| 第5回 | 5-208 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000214 | 2005/7/11 | 8.0 |
| 第5回 | 5-209 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000219 | 2005/7/11 | 8.0 |
| 第5回 | 5-210 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000230 | 2005/7/11 | 8.0 |
| 第5回 | 5-211 | 感染症および寄生虫症 | HIV感染 | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000232 | 2005/7/12 | 8.0 |
| 第5回 | 5-212 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000233 | 2005/7/12 | 8.0 |
| 第5回 | 5-213 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000235 | 2005/7/12 | 8.0 |
| 第5回 | 5-214 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000237 | 2005/7/12 | 8.0 |
| 第5回 | 5-215 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000240 | 2005/7/15 | 8.0 |
| 第5回 | 5-216 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000241 | 2005/7/15 | 8.0 |
| 第5回 | 5-217 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000242 | 2005/7/15 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 一番号 | 感染症の種類 器官別大分類 | 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA(Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-218 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000248 | 2005/7/15 | 8.0 |
| 第5回 | 5-219 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000249 | 2005/7/15 | 8.0 |
| 第5回 | 5-220 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000250 | 2005/7/15 | 8.0 |
| 第5回 | 5-221 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000251 | 2005/7/15 | 8.0 |
| 第5回 | 5-222 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 女性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000252 | 2005/7/19 | 8.0 |
| 第5回 | 5-223 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000254 | 2005/7/19 | 8.0 |
| 第5回 | 5-224 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000256 | 2005/7/19 | 8.0 |
| 第5回 | 5-225 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000257 | 2005/7/22 | 8.0 |
| 第5回 | 5-226 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000258 | 2005/7/22 | 8.0 |
| 第5回 | 5-227 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000259 | 2005/7/22 | 8.0 |
| 第5回 | 5-228 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000261 | 2005/7/22 | 8.0 |
| 第5回 | 5-229 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000264 | 2005/7/22 | 8.0 |
| 第5回 | 5-230 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000267 | 2005/7/22 | 8.0 |
| 第5回 | 5-231 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000274 | 2005/7/26 | 8.0 |
| 第5回 | 5-232 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000276 | 2005/7/26 | 8.0 |
| 第5回 | 5-233 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000279 | 2005/7/26 | 8.0 |
| 第5回 | 5-234 | 感染症および寄生虫症 | HIV感染 | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000297 | 2005/7/26 | 8.0 |
| 第5回 | 5-235 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000299 | 2005/7/26 | 8.0 |
| 第5回 | 5-236 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000302 | 2005/7/26 | 8.0 |
| 第5回 | 5-237 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000282 | 2005/7/26 | 8.0 |
| 第5回 | 5-238 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000283 | 2005/7/26 | 8.0 |
| 第5回 | 5-239 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000284 | 2005/7/26 | 8.0 |
| 第5回 | 5-240 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000285 | 2005/7/26 | 8.0 |
| 第5回 | 5-241 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000286 | 2005/7/26 | 8.0 |
| 第5回 | 5-242 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000287 | 2005/7/26 | 8.0 |
| 第5回 | 5-243 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000288 | 2005/7/26 | 8.0 |
| 第5回 | 5-244 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000292 | 2005/7/26 | 8.0 |
| 第5回 | 5-245 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000293 | 2005/7/26 | 8.0 |
| 第5回 | 5-246 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000295 | 2005/7/26 | 8.0 |
| 第5回 | 5-247 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000296 | 2005/7/26 | 8.0 |
| 第5回 | 5-248 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000305 | 2005/7/26 | 8.0 |
| 第5回 | 5-249 | 感染症および寄生虫症 | HIV感染 | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000308 | 2005/7/26 | 8.0 |
| 第5回 | 5-250 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000314 | 2005/7/27 | 8.0 |
| 第5回 | 5-251 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000315 | 2005/7/27 | 8.0 |
| 第5回 | 5-252 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000316 | 2005/7/27 | 8.0 |
| 第5回 | 5-253 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000317 | 2005/7/27 | 8.0 |
| 第5回 | 5-254 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000320 | 2005/7/27 | 8.0 |
| 第5回 | 5-255 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000321 | 2005/7/27 | 8.0 |
| 第5回 | 5-256 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000334 | 2005/7/29 | 8.0 |
| 第5回 | 5-257 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000335 | 2005/7/29 | 8.0 |
| 第5回 | 5-258 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000342 | 2005/8/2 | 8.0 |
| 第5回 | 5-259 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000350 | 2005/8/2 | 8.0 |
| 第5回 | 5-260 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000353 | 2005/8/2 | 8.0 |
| 第5回 | 5-261 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000356 | 2005/8/2 | 8.0 |
| 第5回 | 5-262 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000358 | 2005/8/2 | 8.0 |
| 第5回 | 5-263 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000360 | 2005/8/2 | 8.0 |
| 第5回 | 5-264 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000368 | 2005/8/3 | 8.0 |
| 第5回 | 5-265 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000383 | 2005/8/3 | 8.0 |
| 第5回 | 5-266 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000387 | 2005/8/3 | 8.0 |
| 第5回 | 5-267 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000390 | 2005/8/8 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 器官別大分類 | 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA(Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-268 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000396 | 2005/8/10 | 8.0 |
| 第5回 | 5-269 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000397 | 2005/8/10 | 8.0 |
| 第5回 | 5-270 | 感染症および寄生虫症 | HIV感染 | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000401 | 2005/8/10 | 8.0 |
| 第5回 | 5-271 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000403 | 2005/8/11 | 8.0 |
| 第5回 | 5-272 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000407 | 2005/8/23 | 8.0 |
| 第5回 | 5-273 | 感染症および寄生虫症 | HIV感染 | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000408 | 2005/8/23 | 8.0 |
| 第5回 | 5-274 | 感染症および寄生虫症 | HIV感染 | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000410 | 2005/8/24 | 8.0 |
| 第5回 | 5-275 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000415 | 2005/8/24 | 8.0 |
| 第5回 | 5-276 | 感染症および寄生虫症 | HIV感染 | パナマ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000422 | 2005/8/30 | 8.0 |
| 第5回 | 5-277 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000423 | 2005/8/30 | 8.0 |
| 第5回 | 5-278 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000424 | 2005/8/30 | 8.0 |
| 第5回 | 5-279 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000425 | 2005/8/30 | 8.0 |
| 第5回 | 5-280 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000427 | 2005/8/30 | 8.0 |
| 第5回 | 5-281 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000428 | 2005/8/30 | 8.0 |
| 第5回 | 5-282 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000429 | 2005/8/30 | 8.0 |
| 第5回 | 5-283 | 感染症および寄生虫症 | HIV感染 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000430 | 2005/8/30 | 8.0 |
| 第5回 | 5-284 | 感染症および寄生虫症 | HIV感染 | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000431 | 2005/8/30 | 8.0 |
| 第5回 | 5-285 | 感染症および寄生虫症 | 後天性免疫不全症候群 | イギリス | 男性 | 不明 | 2002 | 不明 | 症例報告 | 外国製品 | 04000128 | 2005/3/31 | 8.0 |
| 第5回 | 5-115 | 感染症および寄生虫症 | 後天性免疫不全症候群 | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000021 | 2005/5/23 | 8.0 |
| 第5回 | 5-153 | 感染症および寄生虫症 | 後天性免疫不全症候群 | 香港 | 男性 | 4 | 不明 | 不明 | 症例報告 | 外国製品 | 05000113 | 2005/6/9 | 8.0 |
| 第5回 | 5-286 | 肝胆道系障害 | 肝炎 | ブラジル | 男性 | 13 | 不明 | 不明 | 症例報告 | 外国製品 | 05000273 | 2005/7/22 | 8.0 |
| 第5回 | 5-287 | 臨床検査 | B型肝炎ウイルス | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000107 | 2005/3/3 | 7.1 |
| 第5回 | 5-288 | 臨床検査 | B型肝炎ウイルス | イギリス | 男性 | 25 | 不明 | 不明 | 症例報告 | 外国製品 | 05000003 | 2005/4/18 | 8.0 |
| 第5回 | 5-285 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 不明 | 1990 | 不明 | 症例報告 | 外国製品 | 04000128 | 2005/3/31 | 8.0 |
| 第5回 | 5-107 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000012 | 2005/7/29 | 8.0 |
| 第5回 | 5-108 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000013 | 2005/5/12 | 8.0 |
| 第5回 | 5-109 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000015 | 2005/5/23 | 8.0 |
| 第5回 | 5-110 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000016 | 2005/5/23 | 8.0 |
| 第5回 | 5-111 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000017 | 2005/5/23 | 8.0 |
| 第5回 | 5-112 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000018 | 2005/5/23 | 8.0 |
| 第5回 | 5-113 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000019 | 2005/5/23 | 8.0 |
| 第5回 | 5-114 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000020 | 2005/5/23 | 8.0 |
| 第5回 | 5-115 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000021 | 2005/5/23 | 8.0 |
| 第5回 | 5-116 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000022 | 2005/5/23 | 8.0 |
| 第5回 | 5-117 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000023 | 2005/5/23 | 8.0 |
| 第5回 | 5-118 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000025 | 2005/5/26 | 8.0 |
| 第5回 | 5-119 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000026 | 2005/5/26 | 8.0 |
| 第5回 | 5-120 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000027 | 2005/5/26 | 8.0 |
| 第5回 | 5-121 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000053 | 2005/5/30 | 8.0 |
| 第5回 | 5-122 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000054 | 2005/5/30 | 8.0 |
| 第5回 | 5-123 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000056 | 2005/5/30 | 8.0 |
| 第5回 | 5-124 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000057 | 2005/5/30 | 8.0 |
| 第5回 | 5-125 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000058 | 2005/5/30 | 8.0 |
| 第5回 | 5-126 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000061 | 2005/5/30 | 8.0 |
| 第5回 | 5-127 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000062 | 2005/5/30 | 8.0 |
| 第5回 | 5-128 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000063 | 2005/5/30 | 8.0 |
| 第5回 | 5-129 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000064 | 2005/5/30 | 8.0 |
| 第5回 | 5-130 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000065 | 2005/5/30 | 8.0 |
| 第5回 | 5-131 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000066 | 2005/5/30 | 8.0 |
| 第5回 | 5-132 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000067 | 2005/5/30 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA (Ver.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語（PT） | | | | | | | | | | |
| 第5回 | 5-133 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000068 | 2005/5/30 | 8.0 |
| 第5回 | 5-134 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000098 | 2005/6/1 | 8.0 |
| 第5回 | 5-135 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000099 | 2005/6/1 | 8.0 |
| 第5回 | 5-136 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000100 | 2005/6/1 | 8.0 |
| 第5回 | 5-138 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000103 | 2005/6/2 | 8.0 |
| 第5回 | 5-139 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000104 | 2005/6/2 | 8.0 |
| 第5回 | 5-140 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000105 | 2005/6/2 | 8.0 |
| 第5回 | 5-144 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000030 | 2005/5/30 | 8.0 |
| 第5回 | 5-144 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000030 | 2005/6/15 | 8.0 |
| 第5回 | 5-145 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000031 | 2005/5/30 | 8.0 |
| 第5回 | 5-145 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000031 | 2005/6/15 | 8.0 |
| 第5回 | 5-146 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000032 | 2005/5/30 | 8.0 |
| 第5回 | 5-146 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000032 | 2005/6/15 | 8.0 |
| 第5回 | 5-147 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000033 | 2005/5/30 | 8.0 |
| 第5回 | 5-148 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000034 | 2005/5/30 | 8.0 |
| 第5回 | 5-149 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000035 | 2005/5/30 | 8.0 |
| 第5回 | 5-151 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000111 | 2005/6/9 | 8.0 |
| 第5回 | 5-152 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000112 | 2005/6/9 | 8.0 |
| 第5回 | 5-153 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 4 | 不明 | 不明 | 症例報告 | 外国製品 | 05000113 | 2005/6/9 | 8.0 |
| 第5回 | 5-154 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000037 | 2005/5/30 | 8.0 |
| 第5回 | 5-156 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000039 | 2005/5/30 | 8.0 |
| 第5回 | 5-157 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000040 | 2005/5/30 | 8.0 |
| 第5回 | 5-158 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000116 | 2005/6/9 | 8.0 |
| 第5回 | 5-159 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000041 | 2005/5/30 | 8.0 |
| 第5回 | 5-161 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000043 | 2005/5/30 | 8.0 |
| 第5回 | 5-162 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000044 | 2005/5/30 | 8.0 |
| 第5回 | 5-162 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000044 | 2005/6/27 | 8.0 |
| 第5回 | 5-163 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000045 | 2005/5/30 | 8.0 |
| 第5回 | 5-163 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000045 | 2005/6/15 | 8.0 |
| 第5回 | 5-164 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000046 | 2005/5/30 | 8.0 |
| 第5回 | 5-165 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000047 | 2005/5/30 | 8.0 |
| 第5回 | 5-166 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000048 | 2005/5/30 | 8.0 |
| 第5回 | 5-167 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000049 | 2005/5/30 | 8.0 |
| 第5回 | 5-167 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000049 | 2005/6/15 | 8.0 |
| 第5回 | 5-168 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000050 | 2005/5/30 | 8.0 |
| 第5回 | 5-168 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000050 | 2005/6/15 | 8.0 |
| 第5回 | 5-169 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000051 | 2005/5/30 | 8.0 |
| 第5回 | 5-169 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000051 | 2005/6/15 | 8.0 |
| 第5回 | 5-170 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000052 | 2005/5/30 | 8.0 |
| 第5回 | 5-170 | 臨床検査 | C型肝炎ウイルス | 香港 | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000052 | 2005/6/15 | 8.0 |
| 第5回 | 5-173 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000121 | 2005/6/13 | 8.0 |
| 第5回 | 5-174 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000122 | 2005/6/13 | 8.0 |
| 第5回 | 5-177 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000129 | 2005/6/15 | 8.0 |
| 第5回 | 5-180 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000148 | 2005/6/20 | 8.0 |
| 第5回 | 5-181 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000156 | 2005/6/27 | 8.0 |
| 第5回 | 5-182 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000159 | 2005/6/27 | 8.0 |
| 第5回 | 5-185 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000164 | 2005/6/27 | 8.0 |
| 第5回 | 5-186 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000165 | 2005/6/27 | 8.0 |
| 第5回 | 5-187 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000167 | 2005/6/27 | 8.0 |
| 第5回 | 5-187 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000167 | 2005/8/18 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 一番号 | 感染症の種類 器官別大分類 | 感染症の種類 基本語(PT) | 発現国 | 性別 | 年齢(歳) | 発現時期(年/月/日) | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 MedDRA(Ver.) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 第5回 | 5-188 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000171 | 2005/6/30 | 8.0 | |
| 第5回 | 5-189 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000173 | 2005/6/30 | 8.0 | |
| 第5回 | 5-190 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000175 | 2005/6/30 | 8.0 | |
| 第5回 | 5-191 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000176 | 2005/6/30 | 8.0 | |
| 第5回 | 5-192 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000181 | 2005/6/30 | 8.0 | |
| 第5回 | 5-194 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000185 | 2005/7/4 | 8.0 | |
| 第5回 | 5-195 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000186 | 2005/7/4 | 8.0 | |
| 第5回 | 5-196 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000187 | 2005/7/4 | 8.0 | |
| 第5回 | 5-197 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000189 | 2005/7/4 | 8.0 | |
| 第5回 | 5-198 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000190 | 2005/7/4 | 8.0 | |
| 第5回 | 5-200 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000194 | 2005/7/4 | 8.0 | |
| 第5回 | 5-201 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000202 | 2005/7/8 | 8.0 | |
| 第5回 | 5-202 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000203 | 2005/7/8 | 8.0 | |
| 第5回 | 5-203 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000204 | 2005/7/8 | 8.0 | |
| 第5回 | 5-204 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000206 | 2005/7/8 | 8.0 | |
| 第5回 | 5-205 | 臨床検査 | C型肝炎ウイルス | コロンビア | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000209 | 2005/7/8 | 8.0 | |
| 第5回 | 5-207 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000213 | 2005/7/11 | 8.0 | |
| 第5回 | 5-208 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000214 | 2005/7/11 | 8.0 | |
| 第5回 | 5-209 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000219 | 2005/7/11 | 8.0 | |
| 第5回 | 5-210 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000230 | 2005/7/11 | 8.0 | |
| 第5回 | 5-211 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000232 | 2005/7/12 | 8.0 | |
| 第5回 | 5-213 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000235 | 2005/7/12 | 8.0 | |
| 第5回 | 5-215 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000240 | 2005/7/15 | 8.0 | |
| 第5回 | 5-216 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000241 | 2005/7/15 | 8.0 | |
| 第5回 | 5-217 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000242 | 2005/7/15 | 8.0 | |
| 第5回 | 5-218 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000248 | 2005/7/15 | 8.0 | |
| 第5回 | 5-219 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000249 | 2005/7/15 | 8.0 | |
| 第5回 | 5-220 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000250 | 2005/7/15 | 8.0 | |
| 第5回 | 5-221 | 臨床検査 | C型肝炎ウイルス | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000251 | 2005/7/15 | 8.0 | |
| 第5回 | 5-223 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000254 | 2005/7/19 | 8.0 | |
| 第5回 | 5-224 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000256 | 2005/7/19 | 8.0 | |
| 第5回 | 5-225 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000257 | 2005/7/22 | 8.0 | |
| 第5回 | 5-226 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000258 | 2005/7/22 | 8.0 | |
| 第5回 | 5-227 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000259 | 2005/7/22 | 8.0 | |
| 第5回 | 5-228 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000261 | 2005/7/22 | 8.0 | |
| 第5回 | 5-229 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000264 | 2005/7/22 | 8.0 | |
| 第5回 | 5-230 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000267 | 2005/7/22 | 8.0 | |
| 第5回 | 5-231 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000274 | 2005/7/26 | 8.0 | |
| 第5回 | 5-232 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000276 | 2005/7/26 | 8.0 | |
| 第5回 | 5-233 | 臨床検査 | C型肝炎ウイルス | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000279 | 2005/7/26 | 8.0 | |
| 第5回 | 5-234 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000297 | 2005/7/26 | 8.0 | |
| 第5回 | 5-235 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000299 | 2005/7/26 | 8.0 | |
| 第5回 | 5-236 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000302 | 2005/7/26 | 8.0 | |
| 第5回 | 5-237 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000282 | 2005/7/26 | 8.0 | |
| 第5回 | 5-239 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000284 | 2005/7/26 | 8.0 | |
| 第5回 | 5-241 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000286 | 2005/7/26 | 8.0 | |
| 第5回 | 5-242 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000287 | 2005/7/26 | 8.0 | |
| 第5回 | 5-243 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000288 | 2005/7/26 | 8.0 | |
| 第5回 | 5-245 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000293 | 2005/7/26 | 8.0 | |
| 第5回 | 5-249 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000308 | 2005/7/26 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | （備考）MedDRA（Ver.） | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 感染別大分類 | 基本語（PT） | | | | | | | | | | | |
| 第5回 | 5-250 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000314 | 2005/7/27 | 8.0 | |
| 第5回 | 5-251 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000315 | 2005/7/27 | 8.0 | |
| 第5回 | 5-252 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000316 | 2005/7/27 | 8.0 | |
| 第5回 | 5-253 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000317 | 2005/7/27 | 8.0 | |
| 第5回 | 5-254 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000320 | 2005/7/27 | 8.0 | |
| 第5回 | 5-255 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000321 | 2005/7/27 | 8.0 | |
| 第5回 | 5-256 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000334 | 2005/7/29 | 8.0 | |
| 第5回 | 5-258 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000342 | 2005/8/2 | 8.0 | |
| 第5回 | 5-260 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000353 | 2005/8/2 | 8.0 | |
| 第5回 | 5-262 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000358 | 2005/8/2 | 8.0 | |
| 第5回 | 5-265 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000383 | 2005/8/3 | 8.0 | |
| 第5回 | 5-266 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000387 | 2005/8/3 | 8.0 | |
| 第5回 | 5-268 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000396 | 2005/8/10 | 8.0 | |
| 第5回 | 5-269 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000397 | 2005/8/10 | 8.0 | |
| 第5回 | 5-270 | 臨床検査 | C型肝炎ウイルス | ベネズエラ | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000401 | 2005/8/10 | 8.0 | |
| 第5回 | 5-271 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000403 | 2005/8/11 | 8.0 | |
| 第5回 | 5-272 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000407 | 2005/8/23 | 8.0 | |
| 第5回 | 5-273 | 臨床検査 | C型肝炎ウイルス | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000408 | 2005/8/23 | 8.0 | |
| 第5回 | 5-274 | 臨床検査 | C型肝炎ウイルス | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000410 | 2005/8/24 | 8.0 | |
| 第5回 | 5-275 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000415 | 2005/8/24 | 8.0 | |
| 第5回 | 5-277 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000423 | 2005/8/30 | 8.0 | |
| 第5回 | 5-278 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000424 | 2005/8/30 | 8.0 | |
| 第5回 | 5-279 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000425 | 2005/8/30 | 8.0 | |
| 第5回 | 5-280 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000427 | 2005/8/30 | 8.0 | |
| 第5回 | 5-282 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000429 | 2005/8/30 | 8.0 | |
| 第5回 | 5-283 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000430 | 2005/8/30 | 8.0 | |
| 第5回 | 5-284 | 臨床検査 | C型肝炎ウイルス | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000431 | 2005/8/30 | 8.0 | |
| 第5回 | 5-287 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000107 | 2005/3/3 | 7.1 | |
| 第5回 | 5-288 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 25 | 不明 | 不明 | 症例報告 | 外国製品 | 05000003 | 2005/4/18 | 8.0 | |
| 第5回 | 5-289 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 57 | 不明 | 不明 | 症例報告 | 外国製品 | 04000102 | 2005/3/3 | 7.1 | |
| 第5回 | 5-290 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 58 | 不明 | 不明 | 症例報告 | 外国製品 | 04000104 | 2005/3/3 | 7.1 | |
| 第5回 | 5-291 | 臨床検査 | C型肝炎ウイルス | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000120 | 2005/3/18 | 8.0 | |
| 第5回 | 5-292 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000024 | 2005/5/26 | 8.0 | |
| 第5回 | 5-293 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000055 | 2005/5/30 | 8.0 | |
| 第5回 | 5-294 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000059 | 2005/5/30 | 8.0 | |
| 第5回 | 5-295 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000060 | 2005/5/30 | 8.0 | |
| 第5回 | 5-296 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000101 | 2005/6/1 | 8.0 | |
| 第5回 | 5-297 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000108 | 2005/6/7 | 8.0 | |
| 第5回 | 5-298 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000110 | 2005/6/9 | 8.0 | |
| 第5回 | 5-299 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000115 | 2005/6/9 | 8.0 | |
| 第5回 | 5-300 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000117 | 2005/6/9 | 8.0 | |
| 第5回 | 5-301 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000123 | 2005/6/13 | 8.0 | |
| 第5回 | 5-302 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000127 | 2005/6/15 | 8.0 | |
| 第5回 | 5-303 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000128 | 2005/6/15 | 8.0 | |
| 第5回 | 5-304 | 臨床検査 | C型肝炎ウイルス | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000130 | 2005/6/15 | 8.0 | |
| 第5回 | 5-305 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000131 | 2005/6/15 | 8.0 | |
| 第5回 | 5-306 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000132 | 2005/6/15 | 8.0 | |
| 第5回 | 5-307 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000138 | 2005/6/20 | 8.0 | |
| 第5回 | 5-308 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000139 | 2005/6/20 | 8.0 | |
| 第5回 | 5-309 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000140 | 2005/6/20 | 8.0 | |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 識別番号 | 報告日 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語（PT） | | | | | | | | | | MedDRA（Ver.） |
| 第5回 | 5-310 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000141 | 2005/6/20 | 8.0 |
| 第5回 | 5-311 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000142 | 2005/6/20 | 8.0 |
| 第5回 | 5-312 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000143 | 2005/6/20 | 8.0 |
| 第5回 | 5-313 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000146 | 2005/6/20 | 8.0 |
| 第5回 | 5-314 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000149 | 2005/6/27 | 8.0 |
| 第5回 | 5-315 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000151 | 2005/6/27 | 8.0 |
| 第5回 | 5-316 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000152 | 2005/6/27 | 8.0 |
| 第5回 | 5-317 | 臨床検査 | C型肝炎ウイルス | パナマ | | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000153 | 2005/6/27 | 8.0 |
| 第5回 | 5-318 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000154 | 2005/6/27 | 8.0 |
| 第5回 | 5-319 | 臨床検査 | C型肝炎ウイルス | カナダ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000157 | 2005/6/27 | 8.0 |
| 第5回 | 5-320 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000158 | 2005/6/27 | 8.0 |
| 第5回 | 5-321 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000160 | 2005/6/27 | 8.0 |
| 第5回 | 5-322 | 臨床検査 | C型肝炎ウイルス | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000163 | 2005/6/27 | 8.0 |
| 第5回 | 5-323 | 臨床検査 | C型肝炎ウイルス | ドミニカ共和国 | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000166 | 2005/6/27 | 8.0 |
| 第5回 | 5-324 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000170 | 2005/6/30 | 8.0 |
| 第5回 | 5-325 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000172 | 2005/6/30 | 8.0 |
| 第5回 | 5-326 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000174 | 2005/6/30 | 8.0 |
| 第5回 | 5-327 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000177 | 2005/6/30 | 8.0 |
| 第5回 | 5-328 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000178 | 2005/6/30 | 8.0 |
| 第5回 | 5-329 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000179 | 2005/6/30 | 8.0 |
| 第5回 | 5-330 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000180 | 2005/6/30 | 8.0 |
| 第5回 | 5-331 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000184 | 2005/7/4 | 8.0 |
| 第5回 | 5-332 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000188 | 2005/7/4 | 8.0 |
| 第5回 | 5-333 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000192 | 2005/7/4 | 8.0 |
| 第5回 | 5-334 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000193 | 2005/7/4 | 8.0 |
| 第5回 | 5-335 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000197 | 2005/7/8 | 8.0 |
| 第5回 | 5-336 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000198 | 2005/7/8 | 8.0 |
| 第5回 | 5-337 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000199 | 2005/7/8 | 8.0 |
| 第5回 | 5-338 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000200 | 2005/7/8 | 8.0 |
| 第5回 | 5-339 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000201 | 2005/7/8 | 8.0 |
| 第5回 | 5-340 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000205 | 2005/7/8 | 8.0 |
| 第5回 | 5-341 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000207 | 2005/7/8 | 8.0 |
| 第5回 | 5-342 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000208 | 2005/7/8 | 8.0 |
| 第5回 | 5-343 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000210 | 2005/7/8 | 8.0 |
| 第5回 | 5-344 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000211 | 2005/7/8 | 8.0 |
| 第5回 | 5-345 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000216 | 2005/7/11 | 8.0 |
| 第5回 | 5-346 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000217 | 2005/7/11 | 8.0 |
| 第5回 | 5-347 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000220 | 2005/7/11 | 8.0 |
| 第5回 | 5-348 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000222 | 2005/7/11 | 8.0 |
| 第5回 | 5-349 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000224 | 2005/7/11 | 8.0 |
| 第5回 | 5-350 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000229 | 2005/7/11 | 8.0 |
| 第5回 | 5-351 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000236 | 2005/7/12 | 8.0 |
| 第5回 | 5-352 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000247 | 2005/7/15 | 8.0 |
| 第5回 | 5-353 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000255 | 2005/7/19 | 8.0 |
| 第5回 | 5-354 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000260 | 2005/7/22 | 8.0 |
| 第5回 | 5-355 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000265 | 2005/7/22 | 8.0 |
| 第5回 | 5-356 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000266 | 2005/7/22 | 8.0 |
| 第5回 | 5-357 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000268 | 2005/7/22 | 8.0 |
| 第5回 | 5-358 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000269 | 2005/7/22 | 8.0 |
| 第5回 | 5-359 | 臨床検査 | C型肝炎ウイルス | ベネズエラ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000270 | 2005/7/22 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語（PT） | | | | | | | | 識別番号 | 報告日 | MedDRA（Ver.） |
| 第5回 | 5-360 | 臨床検査 | C型肝炎ウイルス | コスタリカ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000271 | 2005/7/22 | 8.0 |
| 第5回 | 5-361 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000275 | 2005/7/26 | 8.0 |
| 第5回 | 5-362 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000277 | 2005/7/26 | 8.0 |
| 第5回 | 5-363 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000278 | 2005/7/26 | 8.0 |
| 第5回 | 5-364 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000298 | 2005/7/26 | 8.0 |
| 第5回 | 5-365 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000300 | 2005/7/26 | 8.0 |
| 第5回 | 5-366 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000301 | 2005/7/26 | 8.0 |
| 第5回 | 5-367 | 臨床検査 | C型肝炎ウイルス | ブラジル | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000303 | 2005/7/26 | 8.0 |
| 第5回 | 5-368 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000304 | 2005/7/26 | 8.0 |
| 第5回 | 5-369 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000280 | 2005/7/26 | 8.0 |
| 第5回 | 5-370 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000289 | 2005/7/26 | 8.0 |
| 第5回 | 5-371 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000290 | 2005/7/26 | 8.0 |
| 第5回 | 5-372 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000291 | 2005/7/26 | 8.0 |
| 第5回 | 5-373 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000294 | 2005/7/26 | 8.0 |
| 第5回 | 5-374 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000306 | 2005/7/26 | 8.0 |
| 第5回 | 5-375 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000307 | 2005/7/26 | 8.0 |
| 第5回 | 5-376 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000309 | 2005/7/27 | 8.0 |
| 第5回 | 5-377 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000313 | 2005/7/27 | 8.0 |
| 第5回 | 5-378 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000318 | 2005/7/27 | 8.0 |
| 第5回 | 5-379 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000322 | 2005/7/27 | 8.0 |
| 第5回 | 5-380 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000323 | 2005/7/27 | 8.0 |
| 第5回 | 5-381 | 臨床検査 | C型肝炎ウイルス | アメリカ | 男性 | 41 | 不明 | 不明 | 症例報告 | 外国製品 | 05000326 | 2005/7/27 | 8.0 |
| 第5回 | 5-382 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000329 | 2005/7/29 | 8.0 |
| 第5回 | 5-383 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000330 | 2005/7/29 | 8.0 |
| 第5回 | 5-384 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000331 | 2005/7/29 | 8.0 |
| 第5回 | 5-385 | 臨床検査 | C型肝炎ウイルス | アルゼンチン | 女性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000332 | 2005/7/29 | 8.0 |
| 第5回 | 5-386 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000333 | 2005/7/29 | 8.0 |
| 第5回 | 5-387 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000336 | 2005/7/29 | 8.0 |
| 第5回 | 5-388 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000337 | 2005/7/29 | 8.0 |
| 第5回 | 5-389 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000338 | 2005/7/29 | 8.0 |
| 第5回 | 5-390 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000339 | 2005/7/29 | 8.0 |
| 第5回 | 5-391 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000343 | 2005/8/2 | 8.0 |
| 第5回 | 5-392 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000344 | 2005/8/2 | 8.0 |
| 第5回 | 5-393 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 死亡 | 症例報告 | 外国製品 | 05000345 | 2005/8/2 | 8.0 |
| 第5回 | 5-394 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000346 | 2005/8/2 | 8.0 |
| 第5回 | 5-395 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000351 | 2005/8/2 | 8.0 |
| 第5回 | 5-396 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000354 | 2005/8/2 | 8.0 |
| 第5回 | 5-397 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000355 | 2005/8/2 | 8.0 |
| 第5回 | 5-398 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000357 | 2005/8/2 | 8.0 |
| 第5回 | 5-399 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000359 | 2005/8/2 | 8.0 |
| 第5回 | 5-400 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000362 | 2005/8/2 | 8.0 |
| 第5回 | 5-401 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000370 | 2005/8/3 | 8.0 |
| 第5回 | 5-402 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000373 | 2005/8/3 | 8.0 |
| 第5回 | 5-403 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000374 | 2005/8/3 | 8.0 |
| 第5回 | 5-404 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000377 | 2005/8/3 | 8.0 |
| 第5回 | 5-405 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000378 | 2005/8/3 | 8.0 |
| 第5回 | 5-406 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000381 | 2005/8/3 | 8.0 |
| 第5回 | 5-407 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000382 | 2005/8/3 | 8.0 |
| 第5回 | 5-408 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000384 | 2005/8/3 | 8.0 |
| 第5回 | 5-409 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000385 | 2005/8/3 | 8.0 |

別紙様式第4

# 感染症発生症例一覧

| 報告回 | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢（歳） | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語（PT） | | | | | | | | 識別番号 | 報告日 | MedDRA（Ver.） |
| 第5回 | 5-410 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000388 | 2005/8/8 | 8.0 |
| 第5回 | 5-411 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000389 | 2005/8/8 | 8.0 |
| 第5回 | 5-412 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000391 | 2005/8/8 | 8.0 |
| 第5回 | 5-413 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000395 | 2005/8/10 | 8.0 |
| 第5回 | 5-414 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000398 | 2005/8/10 | 8.0 |
| 第5回 | 5-415 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000399 | 2005/8/10 | 8.0 |
| 第5回 | 5-416 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000400 | 2005/8/10 | 8.0 |
| 第5回 | 5-417 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000405 | 2005/8/18 | 8.0 |
| 第5回 | 5-418 | 臨床検査 | C型肝炎ウイルス | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000409 | 2005/8/24 | 8.0 |
| 第5回 | 5-419 | 臨床検査 | C型肝炎ウイルス | オーストラリア | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000411 | 2005/8/24 | 8.0 |
| 第5回 | 5-420 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000412 | 2005/8/24 | 8.0 |
| 第5回 | 5-421 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000413 | 2005/8/24 | 8.0 |
| 第5回 | 5-422 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000414 | 2005/8/24 | 8.0 |
| 第5回 | 5-423 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000418 | 2005/8/26 | 8.0 |
| 第5回 | 5-424 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000420 | 2005/8/26 | 8.0 |
| 第5回 | 5-425 | 臨床検査 | C型肝炎ウイルス | ニュージーランド | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000421 | 2005/8/30 | 8.0 |
| 第5回 | 5-426 | 臨床検査 | C型肝炎ウイルス | パナマ | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000426 | 2005/8/30 | 8.0 |
| 第5回 | 5-427 | 臨床検査 | C型肝炎ウイルス | ブラジル | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 05000432 | 2005/8/30 | 8.0 |
| 第5回 | 5-001 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 不明 | 1986/3 | 不明 | 症例報告 | 外国製品 | 04000118 | 2005/3/18 | 8.0 |
| 第5回 | 5-288 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 25 | 1985/10/25 | 不明 | 症例報告 | 外国製品 | 05000003 | 2005/4/18 | 8.0 |
| 第5回 | 5-006 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 不明 | 1985 | 不明 | 症例報告 | 外国製品 | 04000119 | 2005/3/18 | 8.0 |
| 第5回 | 5-287 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 不明 | 1984/2/16 | 不明 | 症例報告 | 外国製品 | 04000107 | 2005/3/3 | 7.1 |
| 第5回 | 5-285 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 不明 | 1983/12 | 不明 | 症例報告 | 外国製品 | 04000128 | 2005/3/31 | 8.0 |
| 第4回 | 4-01 | 感染症および寄生虫症 | A型肝炎 | イギリス | 男性 | 29 | 不明 | 不明 | 症例報告 | 外国製品 | 04000077 | 2004/10/8 | 7.1 |
| 第4回 | 4-01 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 29 | 不明 | 不明 | 症例報告 | 外国製品 | 04000077 | 2004/10/8 | 7.1 |
| 第4回 | 4-02 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000078 | 2004/10/8 | 7.1 |
| 第4回 | 4-03 | 感染症および寄生虫症 | B型肝炎 | イギリス | 男性 | 11 | 不明 | 不明 | 症例報告 | 外国製品 | 04000081 | 2004/10/8 | 7.1 |
| 第4回 | 4-04 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 15 | 1990/7 | 不明 | 症例報告 | 外国製品 | 04000076 | 2004/10/8 | 7.1 |
| 第4回 | 4-01 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 29 | 不明 | 不明 | 症例報告 | 外国製品 | 04000077 | 2004/10/8 | 7.1 |
| 第4回 | 4-02 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000078 | 2004/10/8 | 7.1 |
| 第4回 | 4-03 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 11 | 不明 | 不明 | 症例報告 | 外国製品 | 04000081 | 2004/10/8 | 7.1 |
| 第4回 | 4-05 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 31 | 不明 | 不明 | 症例報告 | 外国製品 | 04000080 | 2004/10/8 | 7.1 |
| 第4回 | 4-06 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 34 | 不明 | 不明 | 症例報告 | 外国製品 | 04000079 | 2004/10/8 | 7.1 |
| 第4回 | 4-07 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 25 | 不明 | 不明 | 症例報告 | 当該製品 | 04000100 | 2005/2/24 | 7.1 |
| 第4回 | 4-08 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 41 | 不明 | 不明 | 症例報告 | 当該製品 | 04000099 | 2005/2/24 | 7.1 |
| 第4回 | 4-09 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 27 | 不明 | 不明 | 症例報告 | 外国製品 | 04000098 | 2005/2/24 | 7.1 |
| 第4回 | 4-10 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 女性 | 22 | 不明 | 不明 | 症例報告 | 外国製品 | 04000095 | 2005/2/21 | 7.1 |
| 第4回 | 4-11 | 感染症および寄生虫症 | C型肝炎 | アメリカ | 男性 | 17 | 不明 | 不明 | 症例報告 | 外国製品 | 04000094 | 2005/2/21 | 7.1 |
| 第4回 | 4-12 | 感染症および寄生虫症 | C型肝炎 | イギリス | 男性 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 04000075 | 2004/10/8 | 7.1 |
| 第4回 | 4-13 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 32 | 1988/8 | 不明 | 症例報告 | 外国製品 | 04000074 | 2004/10/8 | 7.1 |
| 第4回 | 4-06 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 34 | 1985/1/9 | 不明 | 症例報告 | 外国製品 | 04000079 | 2004/10/8 | 7.1 |
| 第4回 | 4-01 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 29 | 1985 | 不明 | 症例報告 | 外国製品 | 04000077 | 2004/10/8 | 7.1 |
| 第4回 | 4-04 | 感染症および寄生虫症 | HIV感染 | イギリス | 男性 | 15 | 1984/12/14 | 不明 | 症例報告 | 外国製品 | 04000076 | 2004/10/8 | 7.1 |
| 第4回 | 4-05 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 31 | 1987 | 不明 | 症例報告 | 外国製品 | 04000080 | 2004/10/8 | 7.1 |
| 第4回 | 4-03 | 臨床検査 | HIV検査陽性 | イギリス | 男性 | 11 | 1981/11/23 | 不明 | 症例報告 | 外国製品 | 04000081 | 2004/10/8 | 7.1 |
| 第3回 | 3-1 | 感染症および寄生虫症 | C型肝炎 | トルコ | 男性 | 44 | 2001/11/22 | 不明 | 症例報告 | 当該製品 | 04000001 | 2004/4/1 | 7.0 |

| | | | | |
|---|---|---|---|---|
| 60249 | 2007/03/30 | バクスター | 乾燥濃縮人血液凝固第Ⅷ因子 | 乾燥人血液凝固第Ⅷ因子 |
| 60250 | 2007/03/30 | バクスター | 乾燥濃縮人血液凝固第Ⅷ因子 | 人血清アルブミン |

別紙様式第4

別紙5

# 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 8-1 | 感染症および寄生虫症 | ブドウ球菌感染 | カナダ | 女 | 50歳 | 2006/10/20 | 不明 | 症例報告 | 外国製品 | 2006/11/14、3-06000024 |
| | 8-2 | 感染症および寄生虫症 | Ｂ型肝炎 | アメリカ | 男 | 46歳 | 2006/7/17 | 不明 | 症例報告 | 外国製品 | 2007/02/20、3-06000030 |
| 第7回 | 7-1 | 臨床検査 | 抗ＨＢｓ抗体陽性 | アメリカ | 男 | 17歳 | 2006/2/18 | 不明 | 症例報告 | 外国製品 | 2006/04/13、3-05000685 |
| | 7-1 | 感染症および寄生虫症 | Ｂ型肝炎 | アメリカ | 男 | 17歳 | 2006/2 | 不明 | 症例報告 | 外国製品 | 2006/04/13、3-05000685 |
| | 7-2 | 感染症および寄生虫症 | Ｂ型肝炎 | カナダ | 女 | 13歳 | 2005/12/1 | 不明 | 症例報告 | 外国製品 | 2006/06/28、3-06000002 |
| | 7-2 | 臨床検査 | Ｂ型肝炎陽性 | カナダ | 女 | 13歳 | 2005/7/22 | 不明 | 症例報告 | 外国製品 | 2006/06/28、3-06000002 |
| 第6回 | 6-1 | 感染症および寄生虫症 | ウエストナイルウイルス感染 | アメリカ | 男 | 43歳 | 不明 | 不明 | 症例報告 | 外国製品 | 2006/02/15、3-05000574 |
| 第5回 | 4-8 | 臨床検査 | Ｂ型肝炎陽性 | アメリカ | 男 | 66歳 | 2005/1/13 | 不明 | 症例報告 | 外国製品 | 2005/03/30、3-04000093 |
| | 3-10 | 感染症および寄生虫症 | Ｃ型肝炎 | アメリカ | 男 | 26歳 | 2002/11/1 | 不明 | 症例報告 | 外国製品 | 2005/06/28、3-04000022 |
| | 2-4 | 感染症および寄生虫症 | Ｃ型肝炎 | アメリカ | 男 | 26歳 | 2002/8/30 | 不明 | 症例報告 | 外国製品 | 2005/03/09、3-03000017 |
| | 4-9 | 臨床検査 | Ｂ型肝炎抗体陽性 | アメリカ | 女 | 32歳 | 不明 | 不明 | 症例報告 | 外国製品 | 2005/03/28、3-04000097 |
| | 3-10 | 感染症および寄生虫症 | Ｂ型肝炎 | アメリカ | 男 | 26歳 | 不明 | 不明 | 症例報告 | 外国製品 | 2005/06/28、3-04000022 |
| 第4回 | 3-1 | 臨床検査 | Ｂ型肝炎抗体陽性 | カナダ | 女 | 56歳 | 2004/3/10 | 不明 | 症例報告 | 外国製品 | 2004/09/17、3-04000007 |
| | 3-3 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/10/10 | 不明 | 症例報告 | 外国製品 | 2004/09/03、3-04000025 |
| | 3-5 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/8/25 | 不明 | 症例報告 | 外国製品 | 2004/08/31、3-04000026 |
| | 3-6 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/6/20 | 不明 | 症例報告 | 外国製品 | 2004/09/03、3-04000027 |
| | 3-8 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/10/01、3-04000028 |
| | 3-10 | 感染症および寄生虫症 | Ｂ型肝炎 | アメリカ | 男 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/09/17、3-04000022 |
| | 3-10 | 感染症および寄生虫症 | Ｃ型肝炎 | アメリカ | 男 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/09/17、3-04000022 |
| | 4-7 | 感染症および寄生虫症 | 単純ヘルペス | アメリカ | 女 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2005/01/12、3-04000089 |
| | 4-8 | 臨床検査 | Ｂ型肝炎陽性 | アメリカ | 不明 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2005/02/02、3-04000093 |
| | 4-9 | 臨床検査 | Ｂ型肝炎抗体陽性 | アメリカ | 女 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2005/02/23、3-04000097 |

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|---|---|---|---|---|
| 70017 | 2007/04/25 | バイエル薬品 | pH4処理酸性人免疫グロブリン | 人免疫グロブリンG |

# 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第3回 | 3-1 | 肝胆道系障害 | 肝炎 | カナダ | 女 | 56歳 | 2004/3/12 | 不明 | 症例報告 | 外国製品 | 2004/05/07、3-04000007 |
| | 3-2 | 臨床検査 | Ｂ型肝炎陽性 | アメリカ | 女 | 48歳 | 2004/3 | 未回復 | 症例報告 | 外国製品 | 2004/06/21、3-04000017 |
| | 3-3 | 臨床検査 | Ｃ型肝炎陽性 | アメリカ | 女 | 48歳 | 2004/3 | 未回復 | 症例報告 | 外国製品 | 2004/06/21、3-04000017 |
| | 3-4 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/10/10 | 不明 | 症例報告 | 外国製品 | 2004/07/20、3-04000025 |
| | 2-5 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 25歳 | 2003/9/26 | 回復 | 症例報告 | 外国製品 | 2004/07/06、3-03000010 |
| | 3-5 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/8/25 | 不明 | 症例報告 | 外国製品 | 2004/08/31、3-04000026 |
| | 3-6 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/6/20 | 不明 | 症例報告 | 外国製品 | 2004/07/20、3-04000027 |
| | 2-3 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/5/26 | 回復 | 症例報告 | 外国製品 | 2004/08/09、3-03000008 |
| | 3-8 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/08/31、3-04000028 |
| | 3-9 | 感染症および寄生虫症 | Ｃ型肝炎 | カナダ | 女 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/05/31、3-04000011 |
| | 3-10 | 感染症および寄生虫症 | Ｂ型肝炎 | アメリカ | 男 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/06/30、3-04000022 |
| | 3-10 | 感染症および寄生虫症 | Ｃ型肝炎 | アメリカ | 男 | 不明 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/06/30、3-04000022 |
| 第2回 | 2-1 | 臨床検査 | ＨＴＬＶ－１検査陽性 | 日本 | 男 | 26歳 | 2003/12 | 不明 | 症例報告 | 当該製品 | 2004/02/26、1-03000112 |
| | 2-2 | 感染症および寄生虫症 | マイコプラズマ感染 | 日本 | 女 | 不明 | 2003/7 | 不明 | 症例報告 | 当該製品 | 2004/01/14、1-03000016 |
| | 2-3 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 不明 | 2003/5 | 不明 | 症例報告 | 外国製品 | 2004/02/23、3-03000008 |
| | 2-4 | 感染症および寄生虫症 | Ｃ型肝炎 | アメリカ | 男 | 26歳 | 2002/8/30 | 不明 | 症例報告 | 外国製品 | 2004/01/13、3-03000017 |
| | 2-5 | 臨床検査 | ＨＴＬＶ－１検査陽性 | カナダ | 女 | 25歳 | 不明 | 不明 | 症例報告 | 外国製品 | 2004/01/05、3-03000010 |

MedDRAバージョン9.1により集計した。
第5回症例番号4-8, 3-10, 2-4, 4-9, 3-10は、それぞれ第4回症例番号、第3回症例番号および第2回症例番号4-8, 3-10, 2-4, 4-9, 3-10において報告したものの追加報告。
第4回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10は，それぞれ第3回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10において報告したものの追加報告。
第4回症例番号3-1の有害事象名は，第3回報告時では「肝炎」であったが追加情報により，「Ｂ型肝炎抗体陽性」に変更された。
第3回症例番号2-5, 2-3は，それぞれ第2回症例番号2-5, 2-3において報告したものの追加報告。
第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

別紙様式第４

別紙（5）

感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発生国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 60 | 2007年1月 | 不明 | 症例報告 | 外国製品 | 識別番号3-06000033　提出日2007年2月23日 |
| | 2 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 男 | 13 | 不明 | 不明 | 症例報告 | 外国製品 | 識別番号3-06000031　提出日2007年2月21日 |
| | 2 | 感染症および寄生虫症 | B型肝炎 | ドイツ | 男 | 13 | 不明 | 不明 | 症例報告 | 外国製品 | 識別番号3-06000031　提出日2007年2月21日 |
| | 2 | 臨床検査 | ウイルス負荷 | ドイツ | 男 | 13 | 不明 | 不明 | 症例報告 | 外国製品 | 識別番号3-06000031　提出日2007年2月21日 |
| 第7回 | 1 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 男 | 37 | 不明 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 感染症および寄生虫症 | B型肝炎 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 臨床検査 | B型肝炎表面抗原陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 臨床検査 | 抗HBs抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 臨床検査 | 抗HBcIgG抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 2 | 臨床検査 | 抗HBe抗体陽性 | ドイツ | 女 | 27 | 2006年6月 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 3 | 感染症および寄生虫症 | C型肝炎 | 米国 | 不明 | 不明 | 2006年 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |
| | 4 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号：3-06000004 提出日：2006/4/25 |
| | 4 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号：3-06000004 追加報告提出日：2006/4/27 |
| | 4 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 63 | 2005年11月 | 不明 | 症例報告 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例<br>識別番号：3-06000004 追加報告提出日：2006/5/18 |

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|---|---|---|---|---|
| 70033 | 2007/04/26 | CSLベーリング | 人免疫グロブリン | 人免疫グロブリン |

別紙様式第4

## 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 感染症および寄生虫症 | サイトメガロウイルス感染 | 日本 | 女 | 1 | 2007/2/6 | ③未回復 | 症例報告 | 当該製品 | 2007/3/2 提出、識別番号 1-06000233 未完了報告 |
| | 2 | 感染症および寄生虫症 | エプスタイン・バーウイルス感染 | 日本 | 女 | 1 | 2007/1/24 | ③未回復 | 症例報告 | 当該製品 | 2007/3/2 提出、識別番号 1-06000234 未完了報告 |
| | 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 79 | 2006/12/13 | ⑥不明 | 症例報告 | 当該製品 | 2006/12/28 提出、識別番号 1-06000203 未完了報告 |
| | 4 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 39 | 2006/11/29 | ③未回復 | 症例報告 | 当該製品 | 2007/1/5 提出、識別番号 1-06000204 未完了報告 |
| | 5 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 9 | 2006/11/27 | ⑥不明 | 症例報告 | 当該製品 | 2006/12/18 提出、識別番号 1-06000192 未完了報告 |
| | 6 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 62 | 2006/11/24 | ③未回復 | 症例報告 | 当該製品 | 2006/12/22 提出、識別番号 1-06000196 未完了報告 |
| | 7 | 感染症および寄生虫症 | サイトメガロウイルス感染 | 日本 | 男 | 1 | 2006/11/15 | ⑤死亡 | 症例報告 | 当該製品 | 2006/12/18 提出、識別番号 1-06000191 未完了報告 |
| | 8 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 3 | 2006/11/2 | ③未回復 | 症例報告 | 当該製品 | 2006/11/30 提出、識別番号 1-06000184 未完了報告 |
| | 9 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 80 | 2006/10/30 | ⑥不明 | 症例報告 | 当該製品 | 2006/11/13 提出、識別番号 1-06000171 未完了報告 |
| | 10 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 34 | 2006/10/27 | ③未回復 | 症例報告 | 当該製品 | 2006/12/22 提出、識別番号 1-06000195 未完了報告 |
| | 11 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 42 | 2006/10/16 | ③未回復 | 症例報告 | 当該製品 | 2006/11/30 提出、識別番号 1-06000183 未完了報告 |
| | 12 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 65 | 2006/9/21 | ③未回復 | 症例報告 | 当該製品 | 2006/10/18 提出、識別番号 1-06000139 未完了報告 |
| | 13 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 34 | 2006/9/21 | ③未回復 | 症例報告 | 当該製品 | 2006/10/11 提出、識別番号 1-06000132 未完了報告 |
| | 14 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 50 | 2006/9/15 | ①回復 | 症例報告 | 当該製品 | 2006/10/11 提出、識別番号 1-06000134 未完了報告 |
| | 15 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 53 | 2006/7/27 | ②軽快 | 症例報告 | 当該製品 | 2006/12/27 提出、識別番号 1-06000200 未完了報告 |
| | 16 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 57 | 2006/7/20 | ②軽快 | 症例報告 | 当該製品 | 2006/9/4 提出、識別番号 1-06000112 未完了報告 |
| | 17 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 82 | 2006/7/7 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/14 提出、識別番号 1-06000114 未完了報告 |
| | 18 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/10/20提出、識別番号1-06000074 取り下げ 第8回症例番号18は前回報告における第7回症例番号32、33において報告したものの追加報告 |
| | 19 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 41 | 2005/5/18 | ②軽快 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000044 完了報告 第8回症例番号19は前々々回報告における第5回症例番号9において報告したものの追加報告 |
| | 20 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 90 | 2005/5/13 | ②軽快 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000039 完了報告 第8回症例番号20は前々々回報告における第5回症例番号12において報告したものの追加報告 |
| | 21 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 91 | 2005/4/7 | ②軽快 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000019 完了報告 第8回症例番号21は前々々回報告における第5回症例番号18において報告したものの追加報告 |
| | 22 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 7 | 2005/3/18 | ⑥不明 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000030 完了報告 第8回症例番号22は前々々回報告における第5回症例番号22において報告したものの追加報告 |
| | 23 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 4 | 2005/3/8 | ②軽快 | 症例報告 | 当該製品 | 2006/9/27提出、識別番号1-05000005 完了報告 第8回症例番号23は前々々回報告における第5回症例番号27において報告したものの追加報告 |

別紙様式第4

## 感染症発生症例一覧

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 24 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 67 | 2005/2/24 | ②軽快 | 症例報告 | 当該製品 | 2006/9/26提出、識別番号1-04000340　完了報告　第8回症例番号24は前々々回報告における第5回症例番号28において報告したものの追加報告 |
| | 25 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 68 | 2005/2/15 | ②軽快 | 症例報告 | 当該製品 | 2006/9/29提出、識別番号1-04000323　完了報告　第8回症例番号25は前々々々回報告における第4回症例番号1において報告したものの追加報告 |
| | 26 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2004/11/16 | ①回復 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000276　完了報告　第8回症例番号26は前々々々回報告における第4回症例番号9において報告したものの追加報告 |
| | 27 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 72 | 2004/10/8 | ⑤死亡 | 症例報告 | 当該製品 | 2007/2/27提出、識別番号1-04000224　完了報告　第8回症例番号27は前々々々回報告における第4回症例番号15において報告したものの追加報告 |
| | 28 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 80 | 2004/10/7 | ①回復 | 症例報告 | 当該製品 | 2006/9/13提出、識別番号1-04000234　完了報告　第8回症例番号28は前々々々回報告における第4回症例番号16において報告したものの追加報告 |
| | 29 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/7/30 | ⑥不明 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000231　完了報告　第8回症例番号29は前々々々回報告における第4回症例番号23において報告したものの追加報告 |
| | 30 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 72 | 2004/6/17 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/19 提出、識別番号 1-04000148 完了報告 |
| | 31 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 59 | 2004/4/16 | ③未回復 | 症例報告 | 当該製品 | 2007/2/13 提出、識別番号 1-04000039 完了報告 |
| | 32 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 55 | 2002/2/8 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/20提出、識別番号1-04000197　完了報告　第8回症例番号32は前々々々回報告における第4回症例番号46において報告したものの追加報告 |
| 第7回 | 32 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/7/20 提出、識別番号 1-06000074 未完了報告 |
| | 33 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/8/29 提出、識別番号 1-06000074 追加報告 |
| 第5回 | 9 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 41 | 2005/05/18 | ③未回復 | 症例報告 | 当該製品 | 2005/06/07 提出、識別番号 1-05000044 未完了報告 |
| | 12 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 90 | 2005/05/13 | ③未回復 | 症例報告 | 当該製品 | 2005/05/27 提出、識別番号 1-05000039 未完了報告 |
| | 18 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 91 | 2005/04/07 | ①回復又は軽快 | 症例報告 | 当該製品 | 2005/04/26 提出、識別番号 1-05000019 未完了報告 |
| | 22 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 7 | 2005/03/18 | ③未回復 | 症例報告 | 当該製品 | 2005/05/13 提出、識別番号 1-05000030 未完了報告 |
| | 27 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 4 | 2005/03/08 | ③未回復 | 症例報告 | 当該製品 | 2005/04/06 提出、識別番号 1-05000005 未完了報告 |
| | 28 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 67 | 2005/02/24 | ①回復又は軽快 | 症例報告 | 当該製品 | 2005/03/23 提出、識別番号 1-04000340 未完了報告 |
| 第4回 | 1 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 68 | 2005/2/15 | ①軽快 | 症例報告 | 当該製品 | 2005/3/2 提出、識別番号 1-04000323 未完了報告 |
| | 9 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2004/11/16 | ③未回復 | 症例報告 | 当該製品 | 2004/12/28 提出、識別番号 1-04000276 未完了報告 |
| | 15 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 72 | 2004/10/8 | ③未回復 | 症例報告 | 当該製品 | 2004/10/27 提出、識別番号 1-04000224 未完了報告 |
| | 16 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 80 | 2004/10/7 | ①回復 | 症例報告 | 当該製品 | 2004/11/10 提出、識別番号 1-04000234 未完了報告 |
| | 23 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/7/30 | ⑧不明 | 症例報告 | 当該製品 | 2004/11/2 提出、識別番号 1-04000231 未完了報告 |
| | 46 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 55 | 2002/2/8 | ③未回復 | 症例報告 | 当該製品 | 2004/9/24 提出、識別番号 1-04000197 未完了報告 |

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| 70034 | 2007/04/27 | 日本赤十字社 | 新鮮凍結人血漿 | 新鮮凍結人血漿 |



MedDRA/J Ver. 10.0J

別紙様式第4

## 感染症発生症例一覧

| 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|
| | 器官別大分類 | 基本語 | | | | | | | | |
| 1 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 17 | 2007/2/1 | ③未回復 | 症例報告 | 当該製品 | 2007/2/21 提出、識別番号 1-06000225 未完了報告 |
| 2 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 67 | 2007/1/19 | ③未回復 | 症例報告 | 当該製品 | 2007/2/6 提出、識別番号 1-06000215 未完了報告 |
| 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 69 | 2007/1/15 | ⑥不明 | 症例報告 | 当該製品 | 2007/1/30 提出、識別番号 1-06000213 未完了報告 |
| 4 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 76 | 2007/1/15 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/15 提出、識別番号 1-06000217 未完了報告 |
| 5 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 64 | 2007/1/11 | ③未回復 | 症例報告 | 当該製品 | 2007/2/22 提出、識別番号 1-06000229 未完了報告 |
| 6 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 2 | 2006/12/18 | ③未回復 | 症例報告 | 当該製品 | 2007/2/6 提出、識別番号 1-06000216 未完了報告 |
| 7 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 64 | 2006/12/15 | ③未回復 | 症例報告 | 当該製品 | 2007/1/10 提出、識別番号 1-06000207 未完了報告 |
| 8 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 70 | 2006/12/14 | ③未回復 | 症例報告 | 当該製品 | 2007/1/10 提出、識別番号 1-06000209 未完了報告 |
| 9 | 感染症および寄生虫症 | 敗血症 | 日本 | 女 | 71 | 2006/12/13 | ②軽快 | 症例報告 | 当該製品 | 2006/12/27 提出、識別番号 1-06000201 未完了報告 |
| 10 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 87 | 2006/12/1 | ②軽快 | 症例報告 | 当該製品 | 2006/12/25 提出、識別番号 1-06000199 未完了報告 |
| 11 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 39 | 2006/11/29 | ③未回復 | 症例報告 | 当該製品 | 2007/1/5 提出、識別番号 1-06000204 未完了報告 |
| 12 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 70 | 2006/11/24 | ②軽快 | 症例報告 | 当該製品 | 2006/12/12 提出、識別番号 1-06000187 未完了報告 |
| 13 | 感染症および寄生虫症 | クレブシエラ感染 | 日本 | 男 | 20 | 2006/11/18 | ⑤死亡 | 症例報告 | 当該製品 | 2006/12/8 提出、識別番号 1-06000186 未完了報告 |
| 14 | 感染症および寄生虫症 | 桿菌感染 | 日本 | 男 | 20 | 2006/11/18 | ⑤死亡 | 症例報告 | 当該製品 | 2006/12/8 提出、識別番号 1-06000186 未完了報告 |
| 15 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 60 | 2006/11/7 | ①回復 | 症例報告 | 当該製品 | 2006/11/22 提出、識別番号 1-06000180 未完了報告 |
| 16 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 54 | 2006/11/5 | ③未回復 | 症例報告 | 当該製品 | 2007/2/22 提出、識別番号 1-06000226 未完了報告 |
| 17 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 58 | 2006/11/2 | ①回復 | 症例報告 | 当該製品 | 2006/11/22 提出、識別番号 1-06000182 未完了報告 |
| 18 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 58 | 2006/11/2 | ①回復 | 症例報告 | 当該製品 | 2006/12/22 提出、識別番号 1-06000182 未完了報告 |
| 19 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 80 | 2006/10/30 | ⑥不明 | 症例報告 | 当該製品 | 2006/11/13 提出、識別番号 1-06000171 未完了報告 |
| 20 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 13 | 2006/10/16 | ③未回復 | 症例報告 | 当該製品 | 2006/12/13 提出、識別番号 1-06000188 未完了報告 |
| 21 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2006/10/3 | ③未回復 | 症例報告 | 当該製品 | 2006/11/1 提出、識別番号 1-06000146 未完了報告 |
| 22 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 64 | 2006/9/26 | ⑥不明 | 症例報告 | 当該製品 | 2006/10/26 提出、識別番号 1-06000143 未完了報告 |
| 23 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 66 | 2006/9/22 | ②軽快 | 症例報告 | 当該製品 | 2006/10/13 提出、識別番号 1-06000136 未完了報告 |
| 24 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2006/9/22 | ⑥不明 | 症例報告 | 当該製品 | 2006/11/6 提出、識別番号 1-06000136 未完了報告 |
| 25 | 感染症および寄生虫症 | 敗血症 | 日本 | 女 | 14 | 2006/9/21 | ②軽快 | 症例報告 | 当該製品 | 2006/10/11 提出、識別番号 1-06000133 未完了報告 |
| 26 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 65 | 2006/9/21 | ③未回復 | 症例報告 | 当該製品 | 2006/10/18 提出、識別番号 1-06000139 未完了報告 |
| 27 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 63 | 2006/8/2 | ②軽快 | 症例報告 | 当該製品 | 2006/10/13 提出、識別番号 1-06000135 未完了報告 |
| 28 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 57 | 2006/7/20 | ②軽快 | 症例報告 | 当該製品 | 2006/9/4 提出、識別番号 1-06000112 未完了報告 |
| 29 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 52 | 2006/7/20 | ③未回復 | 症例報告 | 当該製品 | 2006/9/22 提出、識別番号 1-06000122 未完了報告 |
| 30 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 52 | 2006/7/20 | ③未回復 | 症例報告 | 当該製品 | 2006/11/2 提出、識別番号 1-06000122 未完了報告 |
| 31 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 82 | 2006/7/7 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/14 提出、識別番号 1-06000114 未完了報告 |
| 32 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/10/20提出、識別番号1-06000074 取り下げ<br>第8回症例番号32は前回報告における第7回症例番号8、9において報告したものの追加報告 |





MedDRA/J Ver. 10.0J

別紙様式第4

# 感染症発生症例一覧

第8回

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|---|---|---|---|---|---|---|---|---|---|---|
| 33 | 感染症および寄生虫症 | 敗血症 | 日本 | 男 | 70 | 2006/5/1 | ⑤死亡 | 症例報告 | 当該製品 | 2006/12/5提出、識別番号1-06000023　完了報告　第8回症例番号33は前回報告における第7回症例番号18において報告したものの追加報告 |
| 34 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 90 | 2006/4/26 | ③未回復 | 症例報告 | 当該製品 | 2006/10/26提出、識別番号1-06000024　取り下げ　第8回症例番号34は前回報告における第7回症例番号20、21において報告したものの追加報告 |
| 35 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 59 | 2005/7/1 | ①回復 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000088　完了報告　第8回症例番号35は前々々回報告における第5回症例番号7において報告したものの追加報告 |
| 36 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 59 | 2005/5/18 | ③未回復 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000054　完了報告　第8回症例番号36は前々々回報告における第5回症例番号15において報告したものの追加報告 |
| 37 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 32 | 2005/5/11 | ③未回復 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000038　完了報告　第8回症例番号37は前々々回報告における第5回症例番号17において報告したものの追加報告 |
| 38 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 63 | 2005/3/30 | ③未回復 | 症例報告 | 当該製品 | 2006/9/27提出、識別番号1-05000015　完了報告　第8回症例番号38は前々々回報告における第5回症例番号26において報告したものの追加報告 |
| 39 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 7 | 2005/3/18 | ⑥不明 | 症例報告 | 当該製品 | 2006/12/21提出、識別番号1-05000030　完了報告　第8回症例番号39は前々々回報告における第5回症例番号27において報告したものの追加報告 |
| 40 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2005/3/9 | ③未回復 | 症例報告 | 当該製品 | 2006/9/27提出、識別番号1-05000004　完了報告　第8回症例番号40は前々々回報告における第5回症例番号30において報告したものの追加報告 |
| 41 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 4 | 2005/3/8 | ②軽快 | 症例報告 | 当該製品 | 2006/9/27提出、識別番号1-05000005　完了報告　第8回症例番号41は前々々回報告における第5回症例番号31において報告したものの追加報告 |
| 42 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2005/3/4 | ②軽快 | 症例報告 | 当該製品 | 2006/9/26提出、識別番号1-04000338　完了報告　第8回症例番号42は前々々回報告における第5回症例番号32において報告したものの追加報告 |
| 43 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 67 | 2005/2/24 | ②軽快 | 症例報告 | 当該製品 | 2006/9/26提出、識別番号1-04000340　完了報告　第8回症例番号43は前々々回報告における第5回症例番号35において報告したものの追加報告 |
| 44 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 68 | 2005/2/15 | ②軽快 | 症例報告 | 当該製品 | 2006/9/29提出、識別番号1-04000323　完了報告　第8回症例番号44は前々々々回報告における第4回症例番号1において報告したものの追加報告 |
| 45 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 76 | 2005/1/24 | ②軽快 | 症例報告 | 当該製品 | 2006/9/29提出、識別番号1-04000320　完了報告　第8回症例番号45は前々々々回報告における第4回症例番号2において報告したものの追加報告 |

別紙様式第4

## 感染症発生症例一覧

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|---|---|---|---|---|---|---|---|---|---|---|
| 46 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 62 | 2004/12/21 | ③未回復 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000285　完了報告第8回症例番号46は前々々々回報告における第4回症例番号8において報告したものの追加報告 |
| 47 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/11/29 | ①回復 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000281　完了報告第8回症例番号47は前々々々回報告における第4回症例番号11において報告したものの追加報告 |
| 48 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 68 | 2004/11/18 | ⑤死亡 | 症例報告 | 当該製品 | 2007/2/27提出、識別番号1-04000256　完了報告第8回症例番号48は前々々々回報告における第4回症例番号14において報告したものの追加報告 |
| 49 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 53 | 2004/11/10 | ②軽快 | 症例報告 | 当該製品 | 2006/9/13提出、識別番号1-04000243　完了報告第8回症例番号49は前々々々回報告における第4回症例番号19において報告したものの追加報告 |
| 50 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 73 | 2004/11/5 | ⑤死亡 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000246　完了報告第8回症例番号50は前々々々回報告における第4回症例番号22において報告したものの追加報告 |
| 51 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 49 | 2004/9/14 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000206　完了報告第8回症例番号51は前々々々回報告における第4回症例番号38において報告したものの追加報告 |
| 52 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 81 | 2004/9/4 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/20提出、識別番号1-04000196　完了報告第8回症例番号52は前々々々回報告における第4回症例番号40において報告したものの追加報告 |
| 53 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 80 | 2004/9/1 | ①回復 | 症例報告 | 当該製品 | 2006/9/19提出、識別番号1-04000174　完了報告第8回症例番号53は前々々々回報告における第4回症例番号43において報告したものの追加報告 |
| 54 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 81 | 2004/8/25 | ⑥不明 | 症例報告 | 当該製品 | 2006/9/20提出、識別番号1-04000196　完了報告第8回症例番号54は前々々々回報告における第4回症例番号44において報告したものの追加報告 |
| 55 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 57 | 2004/8/20 | ②軽快 | 症例報告 | 当該製品 | 2007/2/19提出、識別番号1-04000215　完了報告第8回症例番号55は前々々々回報告における第4回症例番号45において報告したものの追加報告 |
| 56 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 35 | 2004/8/19 | ②軽快 | 症例報告 | 当該製品 | 2006/9/20提出、識別番号1-04000192　完了報告第8回症例番号56は前々々々回報告における第4回症例番号47において報告したものの追加報告 |
| 57 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 43 | 2004/8/16 | ②軽快 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000209　完了報告第8回症例番号57は前々々々回報告における第4回症例番号48において報告したものの追加報告 |
| 58 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 55 | 2004/8/10 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000205　完了報告第8回症例番号58は前々々々回報告における第4回症例番号49において報告したものの追加報告 |

別紙様式第4

# 感染症発生症例一覧

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | 59 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 74 | 2004/8/5 | ③未回復 | 症例報告 | 当該製品 | 2006/9/19提出、識別番号1-04000207　完了報告<br>第8回症例番号58は前々々々回報告における第4回症例番号50において報告したものの追加報告 |
| | 60 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 26 | 2004/8/3 | ②軽快 | 症例報告 | 当該製品 | 2006/9/19提出、識別番号1-04000195　完了報告<br>第8回症例番号59は前々々々回報告における第4回症例番号52において報告したものの追加報告 |
| | 61 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/7/30 | ⑥不明 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000231　完了報告<br>第8回症例番号60は前々々々回報告における第4回症例番号53において報告したものの追加報告 |
| | 62 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 45 | 2004/6/11 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/27 提出、識別番号 1-04000103 完了報告 |
| | 63 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 62 | 2004/5/14 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23 提出、識別番号 1-04000124 完了報告 |
| | 64 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 59 | 2004/4/16 | ③未回復 | 症例報告 | 当該製品 | 2007/2/13 提出、識別番号 1-04000039 完了報告 |
| | 65 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 71 | 2004/2/16 | ①回復 | 症例報告 | 当該製品 | 2006/12/26 提出、識別番号 1-06000149 未完了報告 |
| | 66 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 女 | 55 | 2003/8/15 | ①回復 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000177　完了報告<br>第8回症例番号61は前々々々回報告における第4回症例番号79において報告したものの追加報告 |
| | 67 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 62 | 2003/6/18 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/19提出、識別番号1-04000208　完了報告<br>第8回症例番号62は前々々々回報告における第4回症例番号80において報告したものの追加報告 |
| | 68 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 56 | 2001/9/5 | ②軽快 | 症例報告 | 当該製品 | 2006/9/25 提出、識別番号 1-04000144 完了報告 |
| 第7回 | 8 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/7/20 提出、識別番号 1-06000074 未完了報告 |
| | 9 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2006/6/2 | ②軽快 | 症例報告 | 当該製品 | 2006/8/29 提出、識別番号 1-06000074 追加報告 |
| | 18 | 感染症および寄生虫症 | 敗血症 | 日本 | 男 | 70 | 2006/5/1 | ⑤死亡 | 症例報告 | 当該製品 | 2006/5/17 提出、識別番号 1-06000023 未完了報告 |
| | 20 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 90 | 2006/4/26 | ③未回復 | 症例報告 | 当該製品 | 2006/5/17 提出、識別番号 1-06000024 未完了報告 |
| | 21 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 90 | 2006/4/26 | ③未回復 | 症例報告 | 当該製品 | 2006/6/26 提出、識別番号 1-06000024 追加報告 |
| 第5回 | 7 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 59 | 2005/07/01 | ①回復又は軽快 | 症例報告 | 当該製品 | 2005/07/25 提出、識別番号 1-05000088 未完了報告 |
| | 15 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 59 | 2005/05/18 | ③未回復 | 症例報告 | 当該製品 | 2005/06/20 提出、識別番号 1-05000054 未完了報告 |
| | 17 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 32 | 2005/05/11 | ③未回復 | 症例報告 | 当該製品 | 2005/05/25 提出、識別番号 1-05000038 未完了報告 |
| | 26 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 63 | 2005/03/30 | ③未回復 | 症例報告 | 当該製品 | 2005/04/19 提出、識別番号 1-05000015 未完了報告 |
| | 27 | 感染症および寄生虫症 | C型肝炎 | 日本 | 男 | 7 | 2005/03/18 | ③未回復 | 症例報告 | 当該製品 | 2005/05/13 提出、識別番号 1-05000030 未完了報告 |
| | 30 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 65 | 2005/03/09 | ③未回復 | 症例報告 | 当該製品 | 2005/04/06 提出、識別番号 1-05000004 未完了報告 |
| | 31 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 4 | 2005/03/08 | ③未回復 | 症例報告 | 当該製品 | 2005/04/06 提出、識別番号 1-05000005 未完了報告 |
| | 32 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 75 | 2005/03/04 | ⑧不明 | 症例報告 | 当該製品 | 2005/03/18 提出、識別番号 1-04000338 未完了報告 |
| | 35 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 67 | 2005/02/24 | ①回復又は軽快 | 症例報告 | 当該製品 | 2005/03/23 提出、識別番号 1-04000340 未完了報告 |
| | 1 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 68 | 2005/2/15 | ①軽快 | 症例報告 | 当該製品 | 2005/3/2 提出、識別番号 1-04000323 未完了報告 |
| | 2 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 76 | 2005/1/24 | ①軽快 | 症例報告 | 当該製品 | 2005/2/23 提出、識別番号 1-04000320 未完了報告 |
| | 8 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 62 | 2004/12/27 | ③未回復 | 症例報告 | 当該製品 | 2005/1/7 提出、識別番号 1-04000285 未完了報告 |
| | 11 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/11/29 | ①回復 | 症例報告 | 当該製品 | 2005/1/4 提出、識別番号 1-04000281 未完了報告 |

別紙様式第4

## 感染症発生症例一覧

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|---|---|---|---|---|---|---|---|---|---|---|---|
| 第4回 | 14 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 68 | 2004/12/7 | ③未回復 | 症例報告 | 当該製品 | 2004/12/7 提出、識別番号 1-04000256 未完了報告 |
| | 19 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 53 | 2004/11/10 | ①軽快 | 症例報告 | 当該製品 | 2004/12/14 提出、識別番号 1-04000243 未完了報告 |
| | 22 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 73 | 2004/11/5 | ⑤死亡（副作用・感染症によらない） | 症例報告 | 当該製品 | 2004/11/26 提出、識別番号 1-04000246 未完了報告 |
| | 38 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 49 | 2004/9/14 | ③未回復 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号 1-04000206 未完了報告 |
| | 40 | 感染症および寄生虫症 | C型肝炎 | 日本 | 女 | 81 | 2004/9/4 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/24 提出、識別番号 1-04000196 未完了報告 |
| | 43 | 感染症および寄生虫症 | 細菌感染 | 日本 | 女 | 80 | 2004/9/1 | ①回復 | 症例報告 | 当該製品 | 2004/9/13 提出、識別番号 1-04000174 未完了報告 |
| | 44 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 81 | 2004/8/25 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/24 提出、識別番号 1-04000196 未完了報告 |
| | 45 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 57 | 2004/8/20 | ⑧不明 | 症例報告 | 当該製品 | 2004/10/12 提出、識別番号 1-04000215 未完了報告 |
| | 47 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 35 | 2004/8/19 | ③未回復 | 症例報告 | 当該製品 | 2004/9/24 提出、識別番号 1-04000192 未完了報告 |
| | 48 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 43 | 2004/8/16 | ①軽快 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号 1-04000209 未完了報告 |
| | 49 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 55 | 2004/8/10 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号 1-04000205 未完了報告 |
| | 50 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 74 | 2004/8/5 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号 1-04000207 未完了報告 |
| | 52 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 26 | 2004/8/3 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/24 提出、識別番号 1-04000195 未完了報告 |
| | 53 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/7/30 | ⑧不明 | 症例報告 | 当該製品 | 2004/11/2 提出、識別番号 1-04000231 未完了報告 |
| | 79 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 女 | 55 | 2003/8/15 | ①回復 | 症例報告 | 当該製品 | 2004/9/13 提出、識別番号 1-04000177 未完了報告 |
| | 80 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 63 | 2003/6/18 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号 1-04000208 完了報告 |

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| 70035 | 2007/04/27 | 日本赤十字社 | 人血小板濃厚液 | 人血小板濃厚液 |

別紙様式第4

## 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 58 | 2004/8/12 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000185　完了報告<br>第8回症例番号1は前々々々々回報告における第4回<br>症例番号2において報告したものの追加報告 |
| | 2 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/7/30 | ⑥不明 | 症例報告 | 当該製品 | 2007/1/23提出、識別番号1-04000231　完了報告<br>第8回症例番号2は前々々々々回報告における第4回<br>症例番号3において報告したものの追加報告 |
| | 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 62 | 2004/5/14 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23 提出、識別番号 1-04000124 完了報告 |
| | 4 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 71 | 2004/2/16 | ①回復 | 症例報告 | 当該製品 | 2006/12/26 提出、識別番号 1-06000149 未完了報告 |
| | 5 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 56 | 2001/9/5 | ②軽快 | 症例報告 | 当該製品 | 2006/9/25 提出、識別番号 1-04000144 完了報告 |
| 第4回 | 2 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 58 | 2004/08/12 | ⑧不明 | 症例報告 | 当該製品 | 2004/09/21 提出、識別番号 1-04000185 未完了報告 |
| | 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 59 | 2004/07/30 | ⑧不明 | 症例報告 | 当該製品 | 2004/11/02 提出、識別番号 1-04000231 未完了報告 |

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|---|---|---|---|---|
| 70036 | 2007/04/27 | 日本赤十字社 | 洗浄人赤血球浮遊液 | 洗浄人赤血球浮遊液 |



MedDRA/J Ver. 10.0J

別紙様式第4

## 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 女 | 55 | 2003/8/15 | ①回復 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000177　完了報告<br>第8回症例番号1は前々々々々回報告における第4回<br>症例番号6において報告したものの追加報告 |
| | 2 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 55 | 2004/8/10 | ⑥不明 | 症例報告 | 当該製品 | 2007/2/23提出、識別番号1-04000205　完了報告<br>第8回症例番号2は前々々々々回報告における第4回<br>症例番号4において報告したものの追加報告 |
| | 3 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 74 | 2004/8/5 | ③未回復 | 症例報告 | 当該製品 | 2006/9/19提出、識別番号1-04000207　完了報告<br>第8回症例番号3は前々々々々回報告における第4回<br>症例番号5において報告したものの追加報告 |
| | 4 | 感染症および寄生虫症 | 細菌感染 | 日本 | 男 | 82 | 2006/8/21 | ①回復 | 症例報告 | 当該製品 | 2006/9/4　提出、識別番号　1-06000113 未完了報告 |
| | 5 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 3 | 2006/11/2 | ③未回復 | 症例報告 | 当該製品 | 2006/11/30 提出、識別番号　1-06000184 未完了報告 |
| 第4回 | 4 | 感染症および寄生虫症 | B型肝炎 | 日本 | 女 | 55 | 2004/8/10 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号　1-04000205 未完了報告 |
| | 5 | 感染症および寄生虫症 | B型肝炎 | 日本 | 男 | 74 | 2004/8/5 | ⑧不明 | 症例報告 | 当該製品 | 2004/9/29 提出、識別番号　1-04000207 未完了報告 |
| | 6 | 感染症および寄生虫症 | 伝染性紅斑 | 日本 | 女 | 55 | 2003/8/15 | ①回復 | 症例報告 | 当該製品 | 2004/9/13 提出、識別番号　1-04000177 未完了報告 |

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| 70037 | 2007/04/27 | 日本赤十字社 | 白血球除去人赤血球浮遊液 | 白血球除去人赤血球浮遊液 |





MedDRA/J Ver. 10.0J

別紙様式第４

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第２回 | 2-1 | 感染症および寄生虫症 | Ｃ型肝炎 | ドイツ | 男 | 69歳 | 不明 | 不明 | 症例報告 | 外国製品 | 識別番号：03000020　報告日：2004年2月10日<br>MedDRA: Version(6.1) |
| | 2-2 | 臨床検査 | Ｃ型肝炎抗体陽性 | フランス | 女 | 87歳 | 2003/12/18 | 不明 | 症例報告 | 当該製品 | 識別番号：03000022　報告日：2004年4月9日<br>MedDRA: Version(7.0) |
| | 2-3 | 感染症および寄生虫症 | Ｃ型肝炎 | ドイツ | 男 | 4歳 | 1998/6/10 | 不明 | 症例報告 | 外国製品 | 識別番号：04000006　報告日：2004年4月30日<br>MedDRA: Version(7.0) |
| 第１回 | 1-1 | 臨床検査 | Ｃ型肝炎抗体陽性 | 日本 | 男 | 76歳 | 2003/9/19 | 不明 | 症例報告 | 当該製品 | 登録番号：Ａ03-120　（未完了報告 2003年10月3日）<br>MedDRA: Version(6.1) |

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| 70042 | 2007/05/14 | バクスター | 該当なし | ヒト凝固性たん白質（フィブリノゲン加第Ⅹ Ⅲ因子） |
| 70043 | 2007/05/14 | バクスター | 該当なし | 人血清アルブミン |
| 70044 | 2007/05/14 | バクスター | 該当なし | トロンビン |

別紙様式第４

# 感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発現国 | 性別 | 年齢 | 発現時期（年/月/日） | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第８回 | 7-1 | 感染症および寄生虫症 | Ｃ型肝炎 | ドイツ | 男 | 59歳 | 2001/1/3 | 不明 | 症例報告 | 外国製品 | 識別番号：06000023　報告日：2006年12月27日<br>MedDRA: Version(9.0)<br>第７回症例番号7-1において報告したもの追加報告 |
| | 8-1 | 感染症および寄生虫症 | Ｂ型肝炎 | ドイツ | 男 | 72歳 | 不明 | 未回復 | 症例報告 | 外国製品 | 識別番号：06000028　報告日：2007年1月29日<br>MedDRA: Version(9.1) |
| 第７回 | 7-1 | 感染症および寄生虫症 | Ｃ型肝炎 | ドイツ | 男 | 59歳 | 2001/1/3 | 不明 | 症例報告 | 外国製品 | 識別番号：06000023　報告日：2006年11月9日<br>MedDRA: Version(9.0) |
| 第６回 | 0* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| 第５回 | 5-1 | 感染症および寄生虫症 | Ｃ型肝炎 | ドイツ | 男 | 47歳 | 2005年 | 未回復 | 症例報告 | 外国製品 | 識別番号：05000417　報告日：2005年8月26日<br>MedDRA: Version(8.0) |
| 第４回 | 1-1 | 臨床検査 | Ｃ型肝炎抗体陽性 | 日本 | 男 | 76歳 | 2003/9/19 | 不明 | 症例報告 | 当該製品 | 登録番号：A03-120　報告日：2005年4月28日<br>第１回症例番号1-1において報告したもの（未完了報告）の取り下げ報告<br>MedDRA: Version(6.1) |
| 第３回 | 3-1 | 感染症および寄生虫症 | Ｂ型肝炎 | ドイツ | 男 | 55歳 | 2001年 | 不明 | 症例報告 | 外国製品 | 識別番号：04000030　報告日：2004年7月29日<br>MedDRA: Version(7.0) |

＊ 当該調査期間に対象となる感染症報告はなかった。

別紙(5)

別紙様式第４

感染症発生症例一覧

| | 番号 | 感染症の種類 | | 発生国 | 性別 | 年齢 | 発現時期 | 転帰 | 出典 | 区分 | 備考 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 器官別大分類 | 基本語 | | | | | | | | |
| 第8回 | 1 | 臨床検査 | HIV抗体陽性 | 英国 | 男 | 不明 | 1983年 | 28ヶ月 | 症例報告 | 外国製品 | 識別番号3-06000035<br>提出日2007年3月9日 |
| | 1 | 感染症および寄生虫症 | 後天性免疫不全症候群 | 英国 | 男 | 不明 | 1993年 | 28ヶ月 | 症例報告 | 外国製品 | 識別番号3-06000035<br>提出日2007年3月9日 |
| | 1 | 感染症および寄生虫症 | B型肝炎 | 英国 | 男 | 不明 | 1985年 | 28ヶ月 | 症例報告 | 外国製品 | 識別番号3-06000035<br>提出日2007年3月9日 |
| | 1 | 感染症および寄生虫症 | C型肝炎 | 英国 | 男 | 不明 | 1994年 | 28ヶ月 | 症例報告 | 外国製品 | 識別番号3-06000035<br>提出日2007年3月9日 |
| | 1 | 感染症および寄生虫症 | A型肝炎 | 英国 | 男 | 不明 | 1994年 | 28ヶ月 | 症例報告 | 外国製品 | 識別番号3-06000035<br>提出日2007年3月9日 |
| 第7回 | 1 | 感染症および寄生虫症 | C型肝炎 | ドイツ | 女 | 不明 | 不明 | 不明 | その他 | 外国製品 | 弊社血漿分画製剤と原料血漿が同様の可能性のある症例 |

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