資料2

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り扱いとされた。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。

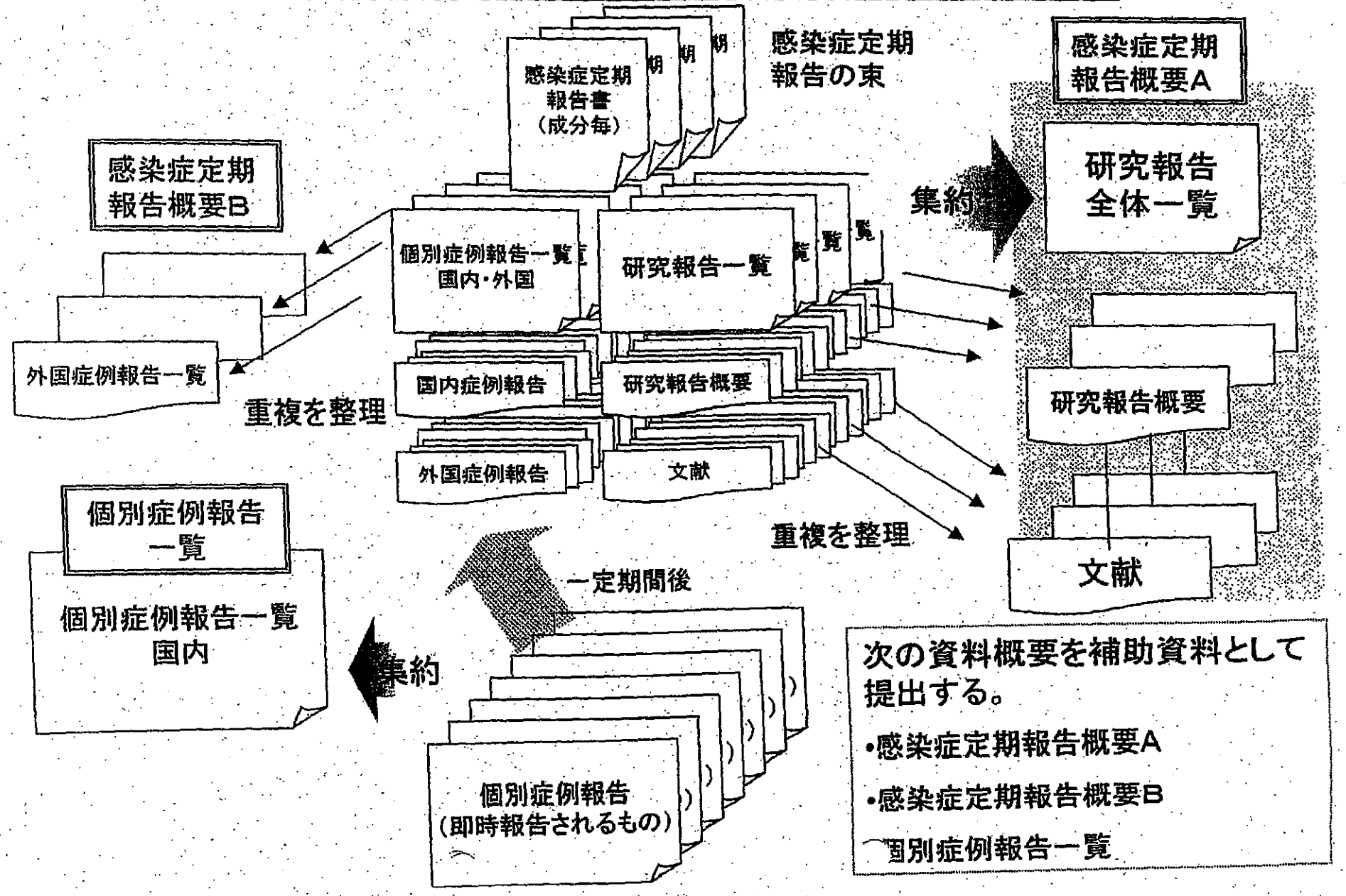
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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、当該症例にかかる「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「感染症的報告について（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

感染症定期報告概要

（平成２３年６月２７日）

平成２２年１２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２２年１２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(2010/12/1～2011/2/28)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100271	2010/12/15	100768	E型肝炎	Lancet. 2010 Sep 11;376(9744):895-902. Epub 2010 Aug 20.	健康成人における組換えE型肝炎ワクチンHEV239(Hecolin; Xiamen Innovax Biotech,Xiamen,China)の有効性及び安全性を多施設共同無作為二重盲検プラセボ対照試験(第Ⅲ相)により評価した。中国の江蘇省で16～65歳の健康成人において、HEV239群とプラセボ群を1:1に無作為割付けを行い、HEV239群にはHEV239ワクチンを、プラセボ群にはB型肝炎ワクチンを0、1、6カ月に1回(全3回)筋注投与し、19カ月間追跡調査された。主要評価項目は3回目の投与後31日目から12カ月の間のE型肝炎の予防効果とされた。参加者はHEV239群(n=56,302)またはプラセボ群(n=56,302)に無作為割付けされ、うち、全3回の接種を受けたHEV239群48,693名(86%)とプラセボ群48,663名(86%)が有効性の一次分析にかけられた。参加者のうち11,165名がE型肝炎ウイルスIgGの検査を受け、そのうち5,285名(47%)が抗体陽性であった。3回目の投与30日後から12カ月間に、プラセボ群の15名がE型肝炎を発症したのに対し、HEV239の有効性は100.0%(95% CI 72.1-100.0)であった。HEV239に関連する重篤な有害事象は示されなかった。以上よりHEV239は、中国の一般集団におけるE型肝炎の予防に有効であると結論づけている。	1
100295	2011/2/22	100916	E型肝炎	Vox Sanguinis 2010,99(Suppl.1) 1-516	血漿分画プールにおけるE型肝炎ウイルス(HEV)の広範囲な分布について、血漿分画プール中のHEV RNAの存在と抗HIV IgG抗体の濃度から検討された。その結果、約10%の血漿分画プールがHEV RNA陽性で、陽性プールの地理的分布はヨーロッパ、北アメリカ、東南アジアの数カ国を含み、広範囲にわたっていた。同定された株の系統発生解析により、genotype4がアジアのプールに限定されていたのに対し、genotype3はヨーロッパと北アメリカのプールで確認された。IgG抗HEV抗体とHEV RNAの間で相関関係は見られず、抗HEV抗体の濃度はアジアのプールでより高かった。以前HEV RNAが日本と中国の供血者で確認されたのに対し、血漿分画プールにおけるHEV RNAの分析はこれまで報告されたことがない。血漿分画製剤のHEVのリスクを判定するために、更なるプールのHEV RNA量を測定する調査が現在行われいる。	2
100268	2010/12/1	100746	レトロウイルス(XMRV)	Proc Natl Acad Sci U S A. 2010 Sep 7;107(36):15874-9.	慢性疲労症候群(CFS)患者と健康な供血者の血液におけるマウス白血病ウイルス(MLV)関連ウイルス遺伝子配列の検出について報告された。最近、末梢血のPCR分析から健康成人218例中8例(3.7%)に対してCFS患者101例中68例(67%)と高率に異種指向性マウス白血病ウイルス関連ウイルス(XMRV)由来DNAが検出されたという研究報告があったが、続く4件の研究報告では、CFS患者の血液から当該DNAは認められていない。今回、CFS診断基準を満たす患者37名からの末梢血単核細胞由来DNA 41検体を調べ、37名中32名にMLV関連ウイルスgag 遺伝子配列を認めたが、健康ボランティア供血者では44名中3名にしか認められなかった。PCR分析システムまたは臨床検体において、マウスDNA混入の証拠は得られていない。全てのXMRVが遺伝的に近縁というこれまでの報告知見に対して、著者らは遺伝的に多様なグループのMLV関連ウイルスを確認した。CFS患者由来のgagおよびenv 配列は、XMRVよりも多指向性マウス内在性レトロウイルスに近縁であり、さらに、同種指向性のMLV配列とは近縁性が低かった。MLV関連ウイルスと同一の強い関連性があるかどうか、これらウイルスがCFS発症において原因的役割を担っているかどうか、ならびにこれらが血液供給の脅威となるかについては、さらなる研究が必要である。	3
100278	2010/12/27	100803	レトロウイルス(XMRV)	ABC Newsletter #31.2010 Aug 27	MLV-related Virusと慢性疲労症候群(CFS)との関連を検討した研究について、及び英国での処置について報告した。PNAS誌では、健康成人からの供血者44名の6.8%において、MLV-like viralの塩基配列が認められたと報告されている。Science誌では、CFS患者101例中68例(67%)にXMRVが検出され、健康成人218例中8例(3.7%)にXMRVが検出されたと報告されている。ABC NewsletterではXMRVは前立腺癌と関連について報告されているが、最新の7月1日のRetrovirologyでは、これまでの結果を確認できなかったとしている。PNAS誌とScience誌の報告に基づいて、11月1日以降、英国ではCFS/ME(筋痛性脳脊髄炎)の罹患歴のある患者由来の献血を永久に延期した。	4

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100271	2010/12/15	100768	パルボウィルス	Transfusion. 2010 Aug;50(8):1712-21	関連供血者と受血者のサンプル分析により確認された、赤血球輸血によるパルボウイルスB19(B19)感染について報告された。受血者のB19 DNAについてはPCR法、B19 IgG抗体についてはEIA法を用いて検査を行った。輸血に関連しない感染を除外するため、B19 DNA陽性受血者の輸血前サンプル及び関連供血者サンプルのB19 DNA、IgG、IgM抗体を検査した。感染の確認のためにDNA配列解析と系統発生解析を行った。その結果、受血者869人中14人(1.6%)がB19 DNA陽性であったが、受血者869人中1人(0.12%)が輸血による感染と確定された。この受血者は、急性感染した供血者からの赤血球(1単位中5×10^{10} IU のB19 DNAを含む)及び他の供血者3名からの赤血球(1,320 IUのB19 IgG抗体を含む)を輸血されていた。感染率(0.12%)は低いが、米国において毎年数百～数千例の感染症が発症する可能性がある。ほとんどの場合無症候性であるが、新生児や免疫不全状態、溶血状態にある者の場合、重篤になることがある。	5
100271	2010/12/15	100768	ウエストナイルウイルス	J Infect Dis. 2010 Nov 1;202(9):1354-61.	供血者スクリーニングによりウイルス血症と特定された者におけるウエストナイル熱の特徴について報告された。2003年6月から2008年にかけて米国赤十字は、初回検査でウエストナイルウイルス(WNV)RNA陽性となった供血者1,436名について、さらに経過観察と追加検査を行ったところ、821名の供血者がWNV感染症であると確認され、残りは未確定または偽陽性であった。WNV感染症の症状を576名の初期WNV感染者と、未感染者の間で比較したところ、WNV感染者の26%に、8症状(新しい発疹、全身の虚脱感、頭痛、重篤な筋肉痛、関節痛、発熱、悪寒、眼痛)のうち少なくとも3症状が存在すると推定された。症状を有する患者の半数近くが治療を求めたが、医療機関の認識不足のためWNV感染の診断を受けた者はわずか5%であった。また、女性および高いウイルス量の者は他の被験者より症状が発現する可能性が高かった。	6
100268	2010/12/1	100746	ウイルス感染	Am J Trop Med Hyg. 2010 Sep;83(3):714-21.	ボリビアとペルーにおけるヒトへのGuaroaウイルス(GROV)感染について報告された。GROVはコロンビアで1959年に初めてヒトから分離された。その後、ブラジル、コロンビア、パナマの発熱患者および蚊からウイルス分離株が採取されたが、ヒトの疾患とウイルスの関連性は不明であった。ボリビアとペルーの発熱疾患患者からGROV14株が分離され、また3症例でIgMセロコンバージョンが確認された。ペルーのIquitos居住者の抗GROV抗体陽性率は13%であり、林業、漁業、油田労働等の就職者において陽性率が高かった。代表的なGROV分離株の遺伝子学的特性からは、ボリビアとペルーの株の特性である単一系統グループを形成しており、以前にブラジルとコロンビアで分離された株とは異なる可能性が示された。本試験で、GROVが中南米の熱帯地域における発熱疾患の原因であることが確認された。	7
100271	2010/12/15	100768	ウイルス感染	Science. 2010 Oct 1;330(6000):20-21.	中国中央部における新型致死性ウイルスの特定について報告された。中国中央部でこの3年間、夏になると数百人が高熱と胃腸障害をきたし、多くの患者が多量出血し、ある地域では患者の30%近くが死亡した。ヒト顆粒球アナプラズマ症が疑われたが、テキサス大学医学部のダニ媒介性疾患の専門家が新型のブニヤウイルスを特定した。その後の研究によりこのウイルスは重症発熱性血小板減少症候群(SFTS)ウイルスと命名され、ブニヤウイルス科フレボウイルス属に分類された。しかしこのウイルスの感染による致死率や、媒介生物はまだ分かっていない。	8

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100268	2010/12/1	100746	HTLV	Blood. 2010 Aug 26;116(8):1211-9	無症候性ヒトT細胞白血病ウイルスⅠ型(HTLV-1)キャリアにおける日本国内の前向き調査:無症候性HTLV-1キャリアにおける成人T細胞白血病(ATL)発症のリスク因子は、現在も不明である。近年、HTLV-1プロウイルス量は、ATLの重要な予測因子として評価されているが、少数の小規模前向き試験が実施されているだけである。2002年～2008年に、登録された無症候性HTLV-1キャ2002年～2008年に、登録された無症候性HTLV-1キャリア1,218名(男性426名、女性792名)を前向きに評価した。リア1,218名(男性426名、女性792名)を前向きに評価した。登録時のプロウイルス量は、女性と比べ男性(末梢血単核細胞(PBMCs)100あたりの中央値1.39 vs 2.10 ; P<0.001)、40歳以下の集団に対し年齢40～49歳および50～59歳の集団(それぞれP = 0.02 , 0.007)、ATL家族歴を有さない集団に対し有する集団(PBMCs 100あたりの中央値1.33 vs 2.32 ; P=0.005)の方が、有意に多かった。14名の登録者が顕在性ATLへ進行した。この登録者の登録時のプロウイルス量は多かった(範囲:PBMCs 100あたり4.17～28.58)。登録時のプロウイルス量が4コピー以下の登録者は、ATLを発症しなかった。また多変量Cox解析では、プロウイルス量だけでなく、高年齢、ATLの家族歴、他疾患治療時における初回HTLV-1検査が、ATLの進行の独立リスク因子であることが示された。	9
100271	2010/12/15	100768	チクングニヤウイルス感染	http://medical.nikkeibp.co.jp/leaf/mem/pub/hotnews/int/201010/516836.html	日本の蚊で伝播する新種ウイルス感染症　チクングニヤ熱が4類感染症に指定された。2010年10月1日、厚生科学審議会感染症分科会感染症部会において、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することが合意された。4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニヤ熱を検疫の対象となる感染症(検疫感染症)に追加することも合意された。チクングニヤ熱は日本国内に広く生息するヒトスジシマカの媒介で伝播する。急性期の患者における血中のウイルス量は多く、当該患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニヤ熱が、国内で広まる可能性は十分高いと危惧している。	10
100295	2011/2/22	100916	新型インフルエンザ(H1N1)	Biologicals 38,2010,652-657	2009年のインフルエンザAウイルスH1N1の世界的流行が未だ猛威をふるっているが、このアウトブレイクによる血漿分画製剤の安全性への影響についてほとんど報告されていない。血漿分画製剤の安全性を評価するため、製造で使用される特定のウイルスクリアランス工程におけるウイルス除去効果が調査された。本研究では、インフルエンザAウイルスH1N1株A/NWS/33(H1N1)をモデルウイルスとした。アルブミンの製造工程では、パスツリゼーションだけでなくフラクションⅣ分画によりH1N1は不活化された。また、静注用免疫グロブリンの製造工程で、フラクションⅢ分画によって沈殿物中にH1N1は除去され、低pHインキュベーションにより完全に不活化された。第Ⅷ因子の製造工程では、0.3%リン酸トリ-n-ブチルと1.0%トリトンX-100を用いたS/D処理で1分以内に完全に不活化され、また98℃乾燥加熱でも10分以内に不活化された。アンチトロンビンⅢの製造工程においても、ViresolveNFPフィルターでH1N1は除去され、パスツリゼーション処理で不活化された。以上の結果から、H1N1は一般に用いられるウイルスクリアランス工程により、効果的に不活化・除去されことがわかった。	11
100268	2010/12/1	100746	デング熱	Am J Trop Med Hyg. 2010 Sep;83(3):664-71.	中国におけるデング熱の報告。中国では大規模アウトブレイクが1978年に既に報告されており、1978から2008年までに計655,324症例(うち610例が死亡)が報告された。1990年代以降、デング熱の流行は、南部沿岸地域から北部や西部にまで拡大している。中国本土において、この20年間でデング熱ウイルスの主要な伝播媒介動物であるヤブカ属の生物学的行動および媒介能が大きく変化してきており、これはおそらく都市化の加速や地球温暖化によるものである。また、人口増加や頻繁な海外旅行もデング熱流行の増加要因となる。デング熱制御への方法は、媒介蚊のコントロール、流行を予測する迅速ウイルス発見システムの確立、地域に密着した教育、そして安全かつ有効なワクチンを開発することである。	12

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100271	2010/12/15	100768	デング熱	ProMED 20100927.3506	アジア地域、オーストラリア、ヨーロッパ、南アメリカのデング熱更新情報について:フィリピンIloilo市では、2010年1月1日～9月18日までに4,825例(死亡27例)。台湾Kaohsiung市では、2010年これまでに227例。ベトナムでは、2010年これまでに55,400例。タイでは、2010年1月～9月11日までに75,852例(死亡87例)。インドネシアJakarta地域では、2010年1月～9月までに1,565例。マレーシアでは2010年これまでに死亡107例(前年死亡70例)。香港では、2010年これまでに43例。ネパールChitwanで7,000例以上の症例(少なくとも死亡19例/月)。インドDelhiでは、95症例以上、蚊媒介性疾患は計2,916例。インドBiharでは陽性518例(死亡6例)。パキスタンKarachiでは、2010年これまでに356例(216例が陽性)。オーストラリア(クイーンズランド州北部)では、4例が陽性、別8例が判定待ち。モナコでは、カリブから戻った18歳居住者に感染が認められた。フランスCorsicaでは、アジア旅行者の3例に症状が発現。フランス(アルプ・マリティーム)では2例。プエルトリコでは、2010年これまでに死亡23例。ホンジュラスでは、2010年これまでに死亡68例。ブラジルRoraimaでは、テング熱4型が12例に増加。ブラジルSao Pauloでは、4,100例(死亡17例)。パラグアイでは、2010年これまでの疑い症例数21,443例、確定症例数が13,678例。	13
100271	2010/12/15	100768	サルモネラ	ProMED 20100915.3343	アイルランドでアヒル卵を摂取することによるサルモネラ症計24例が報告され、アイルランドで近年記録された食中毒の中でも最大規模の流行となっている。感染者の年齢層は生後5カ月～80歳にわたり、最近の症例は裏庭や個人農場から得られたアヒル卵を摂取したことから感染に至っている傾向がある。これを考慮してアイルランド当局では、2010年9月14日、アヒル卵の安全な摂取法に関する助言を行った。また、アヒル卵に触った後の手洗い等の衛生管理を継続することの重要性について警告している。サルモネラネズミチフス菌DT8感染の症状は、嘔吐や下痢による軽度の症状から生命を脅かす疾患に変化してきている。乳児や妊婦、高齢者や病人は最も危険にさらされているため特に注意が必要である。この食中毒の集団発生に鶏卵は関係していない。	14
100276	2010/12/15	100774	コレラ	MMWR Vol. 59	ハイチでコレラのアウトブレークが発生し、2010年10月27日時点で303名の死亡が報告された。2010年10月21日Viblio cholerae 01-serotype Ogawa-biotype E1 Torが同定された。2010年10月27日現在で4,722人が発症し、うち303名に死亡が報告された。ほとんどの症例がArtibonite Departmentで報告されているが、首都のあるQuest Departmentを含む他の地域にも症例が認められている。	15
100268	2010/12/1	100746	細菌感染	Lancet Infect Dis. 2010 Sep;10(9):597-602.	インド、パキスタン、英国での新規抗生物質耐性メカニズムの出現:New Delhi metallo-β-lactamase 1(NDM-1)によるカルバペネム耐性グラム陰性腸内細菌は、世界的に重大な健康問題となる可能性があるため、インド、パキスタン、英国の多剤耐性腸内細菌におけるNDM-1陽性率を調査した。チェンナイ(南インド)、ハリヤーナー(北インド)で腸内細菌の分離株を対象として抗生物質の感受性を評価し、カルバペネム耐性遺伝子blaNDM-1の存在がPCRによって明らかになった。分離株はパルスフィールドゲル電気泳動法で、またプラスミドは、S1ヌクレアーゼ分解およびPCRによって解析された。英国患者については、インドまたはパキスタンへの渡航および最近の入院の有無を調査した。NDM-1産生分離株は、チェンナイ44株、ハリヤーナー26株、英国37株、その他のインド、パキスタン地域で73株が確認された。NDM-1は、ほとんどがEscherichia coli(36株)とKlebsiella pneumoniae(111株)に見つかり、チゲサイクリンとコリスチン以外のの抗生物質に高度耐性があった。ハリヤーナーから分離したK.pneumoniae分離株は遺伝的に均一であったが、英国およびチェンナイの分離株は遺伝的多様性を示した。ほとんどの分離株は、プラスミド上にNDM-1遺伝子を有し、英国およびチェンナイ分離株では受容株に容易にプラスミドが伝達されたが、ハリヤーナーの分離株は伝達性がなかった。英国のNDM-1陽性患者の多くは、前年にインド、パキスタンへの渡航歴があるか、これらの国と関連があった。	16

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No
100268	2010/12/1	100746	細菌感染	www.washingtontimes.com/news/2010/sep/7/japan-confirms-its-first-case-of-new-superbug-gene/	日本初のsuperbug(NDM-1を産生する、新たなタイプの多剤耐性菌)症例が報告された。細菌を薬剤耐性菌に変化させる新たなNew Delhi metallo-β-lactamase 1(NDM-1)遺伝子が日本で初めて、インドで治療を受けた50歳代日本人男性に確認された。この遺伝子はほとんどすべての抗生物質に耐性となるよう細菌を変化させる。この遺伝子は主に病原性大腸菌で見られ、他のタイプの細菌に容易に伝播することが出来るDNA構造を有している。当該男性はインドで内科治療を受け、帰国後の2009年4月に入院した。男性がインドで受けた治療は公表されなかった。男性は入院中に高熱を出したが、2009年10月に退院した。病院はsuperbug(NDM-1を産生する、新たなタイプの多剤耐性菌)を疑い検体を保管、検査し、NDM-1遺伝子の検出について、厚生労働省に届け出た。院内感染は認められていない。日本初のNDM-1症例確認後、厚生労働省は全国調査を開始した。	17
100271	2010/12/15	100768	細菌感染	USA TODAY. Available from: http://www.usatoday.com/yourlife/health/medical/2010-09-17-1Asuperbug17_ST_N.htm	抗生物質の機能を一時的に阻害する酵素Klebsiella pneumoniae *carbapenamase*(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35州を超える病院で報告されている。この細菌は重篤な疾患患者を襲い、死亡率は全症例の30～60%に及ぶ。米国疾病管理予防センター(CDC)によると、New Delhi metallo-β-lactamase 1(NDM-1)は米国では稀であり、KPCの方がはるかに一般的で、現在では米国の半分以上の州で報告されているという。この細菌に対する唯一の薬にポリミキシンがあるが、腎臓に有毒であるため数年前からほとんど使用されていない。従って予防は極めて重要である。2009年3月にCDCは予防に関する新しいガイドラインを示し、特にカルバペネム耐性菌感染症と診断される患者を治療する医師は、ガウンと手袋を着用して自身を守るとともに、他の患者への感染を防がなくてはならないとしている。	18
100271	2010/12/15	100768	感染	MMWR Vol. 59 No. 36	2009年12月14日、ミシシッピー州で、同一ドナーからの腎臓移植レシピエント2名が移植により脳炎を発症した可能性があると米国疾病管理予防センター(CDC)に報告された。CDCはドナーの剖検脳組織からアメーバを発見し、その後、ドナー及びレシピエント2名由来の検体における検査により、バラムチア・アメーバ性肉芽腫性脳炎(GAE)の伝播が確認された。これはBalamuthia mandrillarisに起因する稀な疾患である。レシピエント2名のうち1名(31歳女性)は死亡し、もう1名(27歳男性)は右腕、両脚、視力に後遺症があるが生存している。同一ドナーから心臓移植と肝臓移植を受けたレシピエントには感染の徴候は見られていない。ドナー(4歳)はインフルエンザA感染症を発症後、急性散在性脳脊髄炎で死亡したと推定される。これは臓器移植によるバラムチア感染症の初めての報告である。	19
100278	2010/12/27	100803	異型クロイツフェルト・ヤコブ病	Journal of Pathology 2010;10(1002):2767-2767	英国で採取された扁桃検体におけるリンパ網内性プリオンタンパクの大規模な免疫組織化学検査(IHC)について報告された。英国ではBSE流行の結果、2010年7月5日までにvCJD173例に至っている。扁桃検体63007検体について病因のプリオン蛋白(PrP^{res})に対するEIAを行ったが陰性であった。最もリスクのある1961-1985年生まれの検体とコントロールを含めた別検体について、PrP^{res}、抗プリオン抗体ICMS35及びKG9を用いてIHCを行ったところ、扁桃検体9160中1検体が陽性であり、英国人口100万人あたりプリオン蛋白関連疾患109人の検出率であった。この結果はIHCによってvCJD陽性であるかを見当づけることが出来ると説明している。	20
100278	2010/12/27	100803	異型クロイツフェルト・ヤコブ病	FDA/Vaccines, Blood & Biologics/2010/10/26	米国内採取血漿から製造されたヒト血漿由来第Ⅷ因子(pdFVⅢ)の使用に伴うvCJD罹患リスクとの定量的リスク評価ドラフトの2010年最新版が報告された。pdFVⅢリスク評価モデルで得られた結果から、①米国で製造されたpdFVⅢからのvCJD感染リスクは非常に低いと考えられるが、0ではないこと、②血漿プールがvCJDに汚染されている可能性は低いこと、③vCJD原因物質への曝露の可能性があること、又非常に低いながらも潜在的な感染リスクがあること、④一般的なvCJDリスク、あるいは個々の患者への真のリスクを正確に評価することは不可能であること、⑤感染リスクに影響を及ぼす最も重要な因子が製造ステップでのvCJD原因物質のクリアランス、個々の患者がどの程度の量の製剤を用いるか、及び変数として用いた英国供血者集団におけるvCJDの発生率であることが示唆されている。	21

医薬品 研究報告 調査報告書

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一般的名称	人赤血球濃厚液	研究報告の公表状況	Zhu FC, Zhang J, Zhang XF, Zhou C, Wang ZZ, Huang SJ, Wang H, Yang CL, Jiang HM, Cai JP, Wang YJ, Ai X, Hu YM, Tang Q, Yao X, Yan Q, Xian YL, Wu T, Li YM, Miao J, Ng MH, Shih JW, Xia NS. Lancet. 2010 Sep 11;376(9744):895-902. Epub 2010 Aug 20.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			中国

研究報告の概要

○健常成人における組換えE型肝炎ワクチンの有効性及び安全性:多施設共同無作為二重盲検プラセボ対照試験(第Ⅲ相)
目的:組換えE型肝炎ワクチンHEV239(Hecolin; Xiamen Innovax Biotech,Xiamen,China)の有効性と安全性を、無作為二重盲検プラセボ対照第Ⅲ相試験により評価すること。
方法:中国の江蘇省で16～65歳の健常成人において、HEV239群とプラセボ群を1:1に無作為割付けを行い、HEV239群にはHEV239ワクチンが、プラセボ群にはB型肝炎ワクチンが0、1、6か月に1回ずつ全3回、筋注投与された。参加者は19か月間追跡調査された。プライマリーエンドポイントとして3回目の投与後31日目から12か月の間のE型肝炎の予防効果を観察した。
所見:参加者はHEV239群(n=56,302)またはプラセボ群(n=56,302)に無作為割付けされ、そのうち、問題なく全3回の接種を終えることができたHEV239群48,693名(86%)とプラセボ群48,663名(86%)が有効性の一次分析にかけられた。試験参加者のうち11,165名がE型肝炎ウイルスIgGの検査を受け、そのうち5,285名(47%)が抗体陽性であった。
3回目の投与30日後から12か月間に、15名のプラセボ群参加者がE型肝炎を発症したが、3回の投与後のHEV239の有効性は100.0%(95% CI 72.1-100.0)であった。HEV239に関連する重篤な有害事象は示されなかった。
結論:HEV239は、中国の一般集団におけるE型肝炎の予防に有効である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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
中国で組換えE型肝炎ワクチンHEV239の多施設無作為二重盲検プラセボ対照第Ⅲ相試験を実施したところ、中国の一般集団におけるE型肝炎の予防に有効であったとの報告である。	日本赤十字社では、ALT高値の輸血血液を排除している。また、厚生労働科学研究「経口感染する肝炎ウイルス(A型、E型)の感染防止、遺伝的多様性、および治療に関する研究」班と共同して、献血者におけるHEV感染の疫学調査を行っている。今後もHEV感染の実態に関する情報の収集及び安全対策に努める。

Efficacy and safety of a recombinant hepatitis E vaccine in healthy adults: a large-scale, randomised, double-blind placebo-controlled, phase 3 trial

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Summary

Background Seroprevalence data suggest that a third of the world's population has been infected with the hepatitis E virus. Our aim was to assess efficacy and safety of a recombinant hepatitis E vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China) in a randomised, double-blind, placebo-controlled, phase 3 trial.

Methods Healthy adults aged 16–65 years in, Jiangsu Province, China were randomly assigned in a 1:1 ratio to receive three doses of HEV 239 (30 μg of purified recombinant hepatitis E antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline) or placebo (hepatitis B vaccine) given intramuscularly at 0, 1, and 6 months. Randomisation was done by computer-generated permuted blocks and stratified by age and sex. Participants were followed up for 19 months. The primary endpoint was prevention of hepatitis E during 12 months from the 31st day after the third dose. Analysis was based on participants who received all three doses per protocol. Study participants, care givers, and investigators were all masked to group and vaccine assignments. This trial is registered with ClinicalTrials.gov, number NCT01014845.

Findings *11 165 of the trial participants were tested for hepatitis E virus IgG, of which 5285 (47%) were seropositive for* hepatitis E virus. Participants were randomly assigned to vaccine (n=56 302) or placebo (n=56 302). 48 693 (86%) participants in the vaccine group and 48 663 participants (86%) in the placebo group received three vaccine doses and were included in the primary efficacy analysis. During the 12 months after 30 days from receipt of the third dose 15 per-protocol participants in the placebo group developed hepatitis E compared with none in the vaccine group. Vaccine efficacy after three doses was 100·0% (95% CI 72·1–100·0). Adverse effects attributable to the vaccine were few and mild. No vaccination-related serious adverse event was noted.

Interpretation HEV 239 is well tolerated and effective in the prevention of hepatitis E in the general population in China, including both men and women age 16–65 years.

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Introduction

Hepatitis E virus is a major cause of sporadic and epidemic hepatitis.[1] Seroprevalence data suggest that a third of the world's population has been infected with the virus.[2] Although most cases are in developing countries, hepatitis E is no longer rare and it might be the most common type of acute viral hepatitis in industrialised countries.[3]

Clinically indistinguishable from other types of acute viral hepatitis, hepatitis E tends to be self-limited and usually does not become chronic.[4] The severity of illness increases with age; the overall case fatality ratio is estimated to be 1–3%.[5] Hepatitis E has a poor prognosis in pregnant women: mortality is 5–25%, and survivors have high rates of spontaneous abortion and stillbirth.[6,7] In patients with chronic liver disease, superinfection with hepatitis E virus often leads to a poor outcome.[8,9] Every year, 13 000–26 000 deaths are estimated in patients with chronic liver disease in industrialised countries.[10] In a continuing hepatitis E epidemic in Uganda that has caused illness in more than 10 196 people and 160 deaths, mortality was 13% in children.[11]

At least four genotypes of hepatitis E viruses have been identified.[12] Genotypes 1 and 2 were isolated from human beings and are mainly seen in developing countries. Genotypes 3 and 4 are zoonotic, with pigs being the principal reservoir; they have been identified in many sporadic cases and limited foodborne outbreaks mainly affecting middle-aged and elderly men.[13–15] Nevertheless, all hepatitis E virus associated with human diseases can be considered as belonging to one serotype.[16]

Two recombinant vaccines have undergone phase 2 clinical trials. One of the vaccines was produced in

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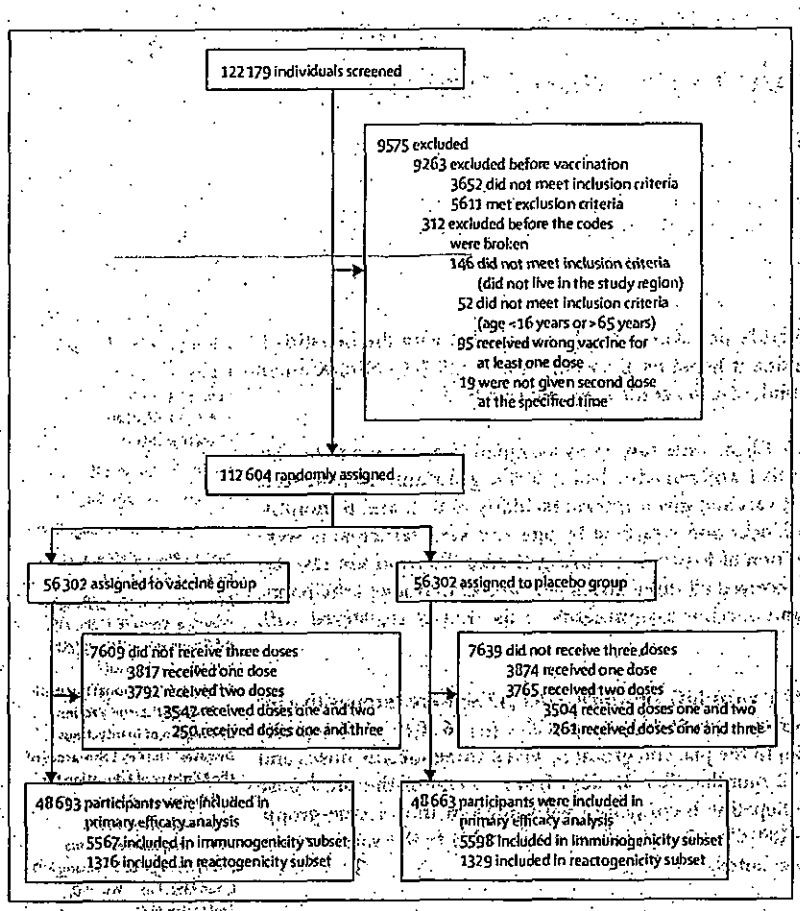

Figure 1: **Trial profile**
The webappendix lists reasons for exclusion (p 30) and for non-completion (p 31).

See Online for webappendix

For the protocol see http://nidvd.xmu.edu.cn/HEV/protocol/Protocol_phase_3_100509.pdf

insect cells and was safe and immunogenic in young men (mean age 25·2 years; SD 6·25), providing 95% protection against hepatitis E in Nepal, where only genotype 1 hepatitis E virus had been isolated.[9] The results were encouraging but two questions remained to be answered. The first related to the safety and efficacy of the vaccine in the general population, especially in women and elderly people. The second related to the efficacy of a vaccine originally derived from hepatitis E virus genotype 1 against disease caused by heterogenic hepatitis E virus. The other candidate vaccine, HEV 239 (Hecolin; Xiamen Innovax Biotech, Xiamen, China), was produced in bacterial cells and was safe and efficacious against infection with hepatitis E virus in seronegative participants in a phase 2 trial.[10]

We undertook a randomised, double-blind, placebo-controlled, phase 3 trial to assess the efficacy and safety of HEV 239 in the general population. The trial included men and women from age 16 to 65 years, with or without antibodies against hepatitis E, from a region where both genotypes 1 and 4 cocirculate with the zoonotic genotype 4 predominating.

Methods

Study design and participants

This double-blind, randomised, placebo-controlled trial was done between August, 2007, and June, 2009, in Dongtai County, Jiangsu Province, China. On October, 2007, after enrolment in one township (Quindong), and before enrolment in ten other townships, the protocol was modified so that each of the 10 000 participants in one of the ten townships (Anfeng) had serum samples collected on day 0 and month 7 to assess the level of antibody protection through long-term follow-up. Independent ethics committee approval was obtained from the Ethics Committee of the Jiangsu Provincial Centre for Disease Control and Prevention (JSCDC), and the study was done in accordance with the principles of the Declaration of Helsinki, the standards of Good Clinical Practice, and Chinese regulatory requirements as stipulated by the Chinese Food and Drug Administration.

The study was designed by JSCDC and Xiamen University. Study staff at JSCDC were responsible for data collection. A sentinel hepatitis surveillance system was set up to identify incident hepatitis cases as they presented. Serial serum samples obtained from study participants were independently tested by the Chinese National Institute for the Control of Pharmaceuticals and Biological Products (NICPBP). A contract research organisation (PPD-Excel PharmaStudies, Beijing, China) monitored and ensured that the trial was done in compliance with the protocol, evaluated progress, verified that the rights of the participants were protected, and ensured that data were complete, accurate, and verifiable from source data. An independent data and safety monitoring board (DSMB) was set up to oversee the trial and ensure the safety of participants and the integrity of the data. The DSMB reviewed the clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment (ie, vaccine *vs* placebo) of trial participants was broken.

Men and women were eligible for enrolment if they were healthy, aged 16–65 years, and understood the study procedures (detailed eligibility criteria are described in webappendix pp 2–3). Written informed consent was obtained from all participants.

Vaccination

The preparation of HEV 239 vaccine is described elsewhere.[10] The vaccine contains 30 μg of the purified antigen adsorbed to 0·8 mg aluminium hydroxide suspended in 0·5 mL buffered saline. A licensed hepatitis B vaccine (Beijing Tiantan Biologic, Beijing, China) containing hepatitis B virus surface antigen in 0·5 mL aluminium hydroxide, was given as placebo. Vaccine doses and placebo doses were repackaged by Innovax under Good Manufacturing Practice conditions for identical appearance, but labelled with two letters each according to a random assignment. Three doses of vaccine or placebo were given intramuscularly at 0, 1, and 6 months.

Randomisation and masking

Trained local health-care workers enrolled the participants, and some of these health-care workers interviewed participants to assess adverse events and possible acute hepatitis later in the trial. An independent statistician prepared a permuted-block 1:1:1:1 randomisation list (with 20 codes to a block) using SAS software. The randomisation list was concealed and transferred into an immunisation management computer program through which participants were stratified by age and sex, and assigned vaccine codes. The study-group and vaccine code assignments were masked from all participants, carers, and investigators (or monitors). The integrity of the masking process was confirmed by the investigators and DSMB before the assignment of study group and vaccine codes was finally revealed. Health-care workers from JSCDC assigned participants to the study groups; they did not have any further involvement in the trial.

A subset of participants from one township was selected for active surveillance of adverse events (reactogenicity subset). Serum samples before immunisation were obtained from these participants and those from another township to establish the baseline concentration of hepatitis E virus IgG and for assessment of immunogenicity (immunogenicity subset). Fingerprint scanners and digital photographs were used to identify and track participants throughout immunisation, blood collection, and follow-up.

	Vaccine group	Placebo group
Randomised participants*	56302	56302
Men	24511 (43·5%)	24567 (43·6%)
Age (years)	44·14 (11·40)	44·13 (11·40)
Age group (years)		
16–20	2520 (4%)	2480 (4%)
21–30	4598 (8%)	4653 (8%)
31–40	12684 (23%)	12688 (23%)
41–50	18292 (32%)	18310 (33%)
51–60	14644 (26%)	14657 (26%)
61–65	3564 (6%)	3514 (6%)
Per-protocol population† (three doses)	48693	48663
Male to female ratio	0·74	0·74
Mean age (years)	44·72, SD 11·09	44·68, SD 11·10
Immunogenicity subset‡	5567	5598
Male to female ratio	0·64	0·65
Mean age (years)	45·22 (10·75)	45·25 (10·82)
Anti-HEV prevalence	47·76% (46·44–49·09)	46·91% (45·60–48·23)
GMC (Wu/mL)	0·14 (0·13–0·14)	0·13 (0·13–0·14)
Reactogenicity subset§	1316	1329
Men	524 (39·8%)	561 (42·2%)
Age (years)	44·70, SD 11·23	44·93, SD 11·10

Data are number (%), mean (SD), or mean (95% CI). HEV=hepatitis E virus. GMC=geometric mean concentration. *All randomised participants who received at least one dose of vaccine or placebo. †Per-protocol population denotes all randomised participants who received three doses of vaccine or placebo. ‡Participants in the immunogenicity subset were from two townships additionally investigated for antibody response to vaccination. §Participants in the reactogenicity subset were from one township visited regularly at home by investigators to assess adverse events.

Table 1: Baseline characteristics of participants

Hepatitis surveillance

Participants with suspected hepatitis were identified through an established active hepatitis surveillance system comprising 205 sentinels, including 162 community clinics, 30 private clinics, 11 central hospitals located in the townships, and two central hospitals in the city of Dongtai (webappendix p 32). A case of hepatitis was defined as a patient presenting with constitutional symptoms such as fatigue, loss of appetite, or both for longer than 3 days with alanine aminotransferase (ALT) exceeding 2·5-times the upper limit of normal range. Patients with abnormal concentrations of ALT were tested at first presentation by JSCDC for hepatitis A virus IgM, surface antigen of hepatitis B virus, hepatitis B virus core protein IgM, hepatitis C virus immunoglobulin, and hepatitis E virus IgM. Paired serum samples were obtained from these patients at the time of presentation and 2–6 weeks later. Serial samples were sent to the NICPBP to test for hepatitis E virus IgM and IgG, hepatitis E virus RNA, and hepatitis A virus IgM. The DSMB reviewed the clinical and laboratory results and confirmed the diagnoses of hepatitis E before unblinding. To be defined as an acute hepatitis E patient, a participant needed to fulfil three conditions: acute illness lasting for at least 3 days; abnormal serum ALT concentration 2·5-times the upper limit of normal range or greater; and positive hepatitis E virus IgM and RNA, ≥4-times increase in hepatitis E virus IgG, or both.

Laboratory measurements

The tests for hepatitis E virus IgM were done by use of two commercial assays in parallel (Beijing Wantai, Beijing, China; MP Biomedicals, Singapore).[11,20–23] The assay for hepatitis E virus IgG used antigen more truncated than that in the vaccine antigen (Beijing Wantai, China).[20,21] Hepatitis E virus IgGs were further quantified and expressed in WHO units per mL (Wu/mL; webappendix p 2). The lower limit of hepatitis E virus IgG quantification was 0·077 Wu/mL.[24] For the analysis, the antibody concentration in samples negative for hepatitis E virus IgG were arbitrarily set at 0·0385 Wu/mL. Serum samples of patients with detectable hepatitis E virus IgM or a two times or greater rise of hepatitis E virus IgG concentration in paired samples were tested for hepatitis E virus RNA.[25] Serum samples were taken before the first vaccine dose and 1 month after the third dose from participants in the immunogenicity subset to establish concentration of hepatitis E virus IgG. Antibody concentration of 0·077 Wu/mL or greater was deemed to be a positive finding. Antibody response was defined as a greater than four-times increase of hepatitis E virus IgG in an individual's paired sera. All reagents were supplied by Beijing Wantai Biological Pharmacy Enterprise, Beijing, China.

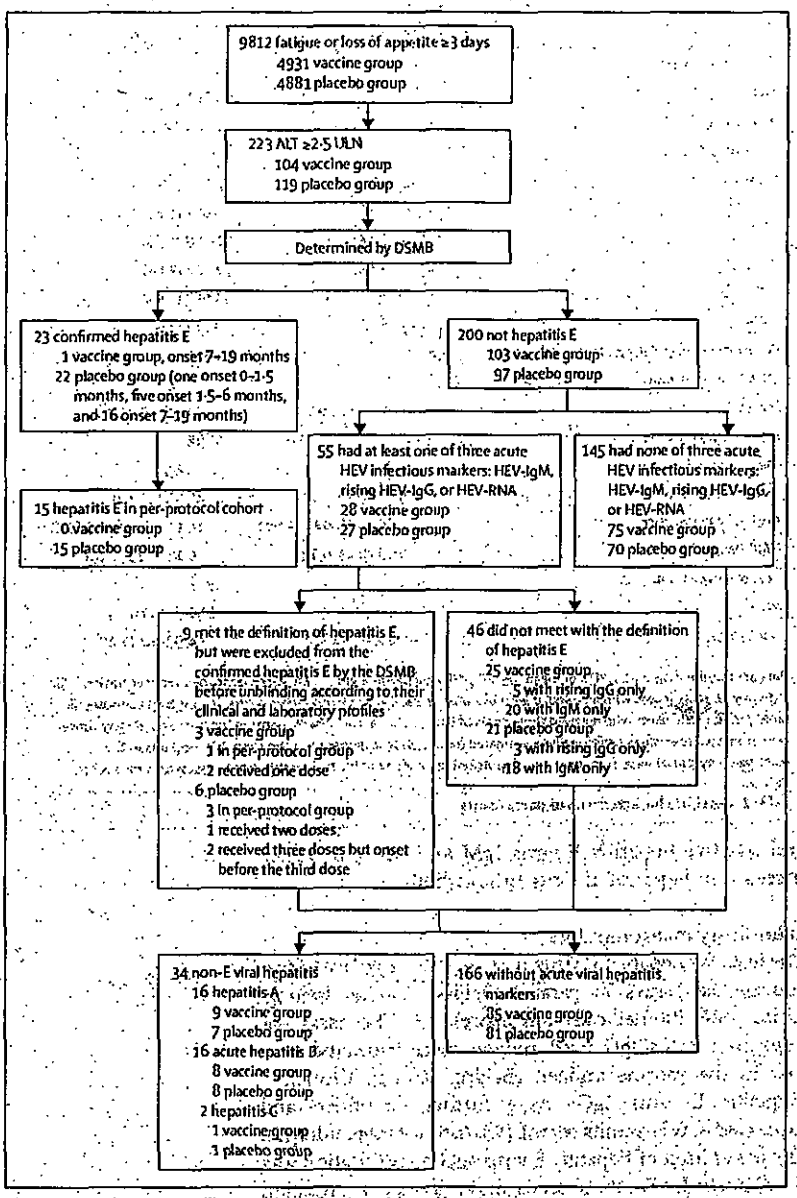

Figure 2: **Flowchart of surveillance and certification of acute hepatitis E**
Sentinel hepatitis surveillance system was set up to monitor study participants for development of acute hepatitis (webappendix p 32). A case of acute hepatitis was defined as a participant who presented with constitutional signs, such as fatigue, nausea for at least 3 days, and alanine aminotransferase (ALT) exceeding 2·5-times the upper limit of normal range (ULN). Clinical and laboratory findings were assessed by an independent data and safety monitoring board (DSMB). The DSMB reviewed clinical and laboratory data to confirm the diagnosis of hepatitis E before the group assignment of trial participants was broken. HEV=hepatitis E virus.

For more on the Medical Dictionary for Regulatory Activities see http://www.meddramsso.com/

Adverse events

After each dose, participants were observed for 30 min for immediate adverse reactions. Participants in the reactogenicity subset were visited at home by investigators at 6 h, 24 h, 48 h, 72 h, 7 days, 14 days, and 28 days after each dose, and observed or reported adverse effects, if any, were recorded on safety diary cards. Other participants were asked to report any adverse events to nearby clinics within 1 month after each dose. Additionally, investigators reviewed all records of admission to hospital and death to identify trial participants. Any serious adverse events were recorded throughout the study by use of the Medical Dictionary for Regulatory Activities (version 12.0).

Statistical analysis

We estimated that the incidence of hepatitis E for adults aged 16–65 years would be about four cases per 10 000 person-years (webappendix p 1). On the assumption of a vaccine efficacy of 70%, a two-group continuity-corrected χ^2 test with a one-sided significance level of 0·05 would have a power of 80% to detect a difference in incidence with 41 277 participants per group. To compensate for dropouts, 50 000 participants per group were needed.

Prespecified outcome analyses were done in eligible participants who had received at least one dose of either vaccine, and in those who received all of the three doses of the vaccines. The primary endpoint was prevention of hepatitis E in participants who received three doses of vaccine (ie, the per-protocol population) during the 12 months from the 31st day after receipt of the third dose. Vaccine efficacy and 95% CIs were calculated on the basis of the identified difference between the vaccine group and the placebo group and the accrued person-time. An exact conditional procedure was used to evaluate vaccine efficacy under the assumption that the numbers of patients with hepatitis E in the vaccine and placebo groups were independent Poisson random variables.[25] For robustness, efficacy was also assessed by use of a Cox proportional hazard model, and a log-rank test was used to compare the cumulative incidence of hepatitis E between the study groups.

Adverse events were summarised for all vaccination visits as frequencies and percentages according to study group. Proportions of events and 95% CIs (unadjusted for multiplicity) were compared between the groups by use of two-sided Fisher's exact test.

Data analysis was done with SAS software version 9.1. All reported p values are two-sided with an α value of 0·05.

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

122 179 people from 11 townships attended the enrolment visit between August and October, 2007. 112 604 participants fulfilled the eligibility requirements, were randomly assigned to the study group, and received at least one dose of vaccine or placebo. 97 356 participants

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	Follow-up (month of study)	Vaccine group			Placebo group			Vaccine efficacy (95% CI)	p value
		Number of participants/ person-years at risk	Number of cases	Incidence (per 10 000 person-years)	Number of participants/ person-years at risk	Number of cases	Incidence (per 10 000 person-years)		
Per-protocol									
Whole group (three doses)	7–19	48 693/48 594·6	0	0·0	48 663/48 555·1	15	3·1	100·0% (72·1–100·0)	<0·0001
Men	7–19	20 662/20 616·1	0	0·0	20 709/20 660·0	11	5·3	100·0% (60·1–100·0)	0·001
Women	7–19	28 031/27 978·5	0	0·0	27 954/27 895·1	4	1·4	100·0% (−51·0 to 100·0)	0·045
Age 16–49 years	7–19	30 374/30 299·5	0	0·0	30 355/30 276·9	6	2·0	100·0% (15·13–100·0)	0·014
Age 50–65 years	7–19	18 319/18 295·2	0	0·0	18 308/18 278·2	9	4·9	100·0% (49·4–100·0)	0·003
First 6 months of follow-up	7–13	48 693/23 981·9	0	0·0	48 663/23 965·8	6	2·5	100·0% (15·12–100·0)	0·014
Second 6 months of follow-up	14–19	48 693/24 612·8	0	0·0	48 663/24 589·3	9	3·7	100·0% (49·4–100·0)	0·003
First two doses subset	1·5–5	54 986/20 202·1	0	0·0	54 973/20 196·8	5	2·5	100·0% (9·1–100·0)	<0·0001
Population receiving at least one dose	7–19	56 302/56 104·7	1	0·2	56 302/56 081·2	16	2·9	93·8% (59·8–99·9)	0·0001
Modified subset one (all participants received at least one dose)	0–19	56 302/87 354·2	1	0·1	56 302/87 323·2	22	2·5	95·5% (66·3–99·4)	<0·0001
Modified subset two (participants in reactogenicity subset were excluded because of lacking follow-up data during 0–6 months)	0–19	54 986/86 040·4	1	0·1	54 973/86 003·4	21	2·4	95·2% (64·6–99·4)	<0·0001

Person-years at risk is the cumulative follow-up years of at risk participants at the indicated timepoint. Number of at risk participants is the initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had dropped out of study).

Table 2: Efficacy of recombinant hepatitis E vaccine.

received all three doses of vaccine or placebo and were included in the analysis of the primary endpoint (figure 1). Table 1 shows the baseline characteristics of study participants.

The DSMB confirmed 23 cases of hepatitis E before unblinding (figure 2); details of each case are listed in webappendix pp 4–24. Compared with the general study population, patients with hepatitis E were older (mean 51·3 years, SD 8·2, median 53, range 36–63) and more likely to be men (male-to-female ratio 2·3). The mean maximum serum ALT concentration of patients with hepatitis E was 30·8 times upper limit of normal range (SD 29·3; 18·1, 2·5–96·9), and the mean duration of illness was 57·1 days (SD 39·8; 41, 9–175). 15 patients were admitted to hospital for a mean of 24·4 days (SD 14·5; 20, 9–66). All 23 patients tested positive for hepatitis E virus IgM, 22 were positive for hepatitis E virus RNA, and 14 had a 4-times or greater increase in hepatitis E virus IgG. Of the 13 patients whose viruses were isolated for sequencing, 12 had genotype 4 and one had genotype 1. Of the eight patients who received all three doses and whose viruses underwent sequencing, all had genotype 4.

In the primary analysis population 15 participants developed hepatitis E during the 12 months from the 31st day after receipt of the third dose; all 15 were in the placebo group (table 2). Vaccine efficacy against hepatitis E was 100·0% (95% CI 72·1–100·0), and protection extended to all participants throughout the 12 months. Five participants developed hepatitis E during the 14 days after the second dose and before the third dose; all were in the placebo group. Vaccine efficacy after two doses was 100·0% (9·1–100·0).

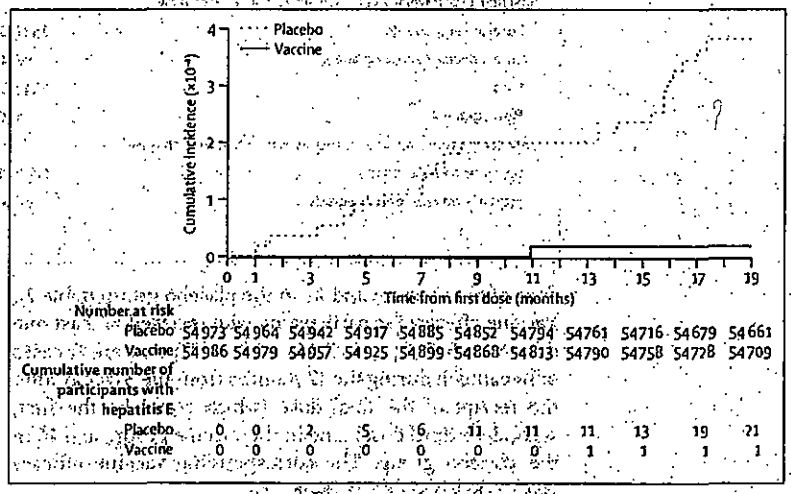

Figure 3: Cumulative incidence of hepatitis E
Cumulative incidence in each group at an indicated time–(cumulative number of cases/cumulated following-up time for at risk participants×10 000). Number at risk–initial number of participants entered in the study–(cumulative hepatitis E cases+participants who had subsequently dropped out of study). The difference between the groups was significant (p<0·001 by log-rank test).

Most randomised participants who received at least one dose of vaccine or placebo were followed up for 19 months from the beginning of the study, and a small proportion of participants were followed up from month 7 of the study. There were 23 cases of hepatitis E during the follow-up, one in the vaccine group (the participant received one

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	Number of adverse events (rate, 95% CI)		p value*
	Vaccine group	Placebo group	
Reactogenicity subset			
Number of participants who received more than one dose	1316	1329	..
Solicited local adverse events within 72 h after each dose			
Local adverse events	177 (13·5%, 11·65–15·41)	94 (7·1%, 5·75–8·59)	<0·0001
Local adverse events ≥grade 3	2 (0·2%, 0·02–0·55)	0 (0·0%, 0·00–0·28)	0·248
Pain	136 (10·3%, 8·74–12·11)	73 (5·5%, 4·33–6·86)	<0·0001
Pain ≥grade 3	0 (0·0%, 0·00–0·28)	0 (0·0%, 0·00–0·28)	..
Swelling	30 (2·3%, 1·54–3·24)	8 (0·6%, 0·26–1·18)	<0·0001
Swelling ≥grade 3	2 (0·2%, 0·02–0·55)	1 (0·0%, 0·00–0·28)	0·248
Itch	20 (1·5%, 0·93–2·34)	13 (1·0%, 0·52–1·67)	0·210
Itch ≥grade 3	0 (0·0%, 0·00–0·28)	0 (0·0%, 0·00–0·28)	..
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	267 (20·3%, 18·15–22·56)	263 (19·8%, 17·68–22·03)	0·748
Systemic adverse events ≥grade 3	7 (0·5%, 0·21–1·09)	4 (0·3%, 0·08–0·77)	0·356
Fever	245 (18·6%, 16·55–20·83)	239 (18%, 15·95–20·16)	0·674
Fever ≥grade 3	6 (0·5%, 0·17–0·99)	3 (0·2%, 0·05–0·66)	0·341
Headache	14 (1·1%, 0·58–1·78)	8 (0·6%, 0·26–1·18)	0·191
Headache ≥grade 3	1 (0·1%, 0·00–0·42)	0 (0·0%, 0·00–0·28)	0·498
Fatigue	28 (2·1%, 1·42–3·06)	20 (1·5%, 0·92–2·31)	0·230
Fatigue ≥grade 3	1 (0·1%, 0·00–0·42)	0 (0·0%, 0·00–0·28)	0·498
Total vaccinated cohort minus the reactogenicity subset			
Number of participants who received more than one dose	54 986	54 973	..
Solicited local adverse events within 72 h after each dose			
Local adverse events	1532 (2·8%, 2·65–2·93)	1051 (1·9%, 1·8–2·03)	<0·0001
Local adverse events ≥grade 3	61 (0·1%, 0·08–0·14)	27 (0·1%, 0·03–0·07)	<0·0001
Pain	1143 (2·1%, 1·96–2·20)	754 (1·4%, 1·28–1·47)	<0·0001
Pain ≥grade 3	1 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	1·000
Solicited systemic adverse events within 72 h after each dose			
Systemic adverse events	1068 (1·9%, 1·83–2·06)	1045 (1·9%, 1·79–2·02)	0·617
Systemic adverse events ≥grade 3	60 (0·1%, 0·08–0·14)	63 (0·1%, 0·09–0·15)	0·786

(Continues on next page)

dose of the vaccine) and 22 in the placebo group (table 2). Vaccine efficacy for participants who received at least one dose was 95·5% (95% CI 66·3–99·4). There were 17 cases of hepatitis E during the 12 months from the 31st day after the receipt of the final dose (which could be the first, second, or third dose), one in the vaccine group, and 16 in the placebo group. The corresponding vaccine efficacy was 93·8% (95% CI 59·8–99·9%).

Figure 3 shows the cumulative incidence of hepatitis E in participants who were followed up for 19 months from the beginning of the study. The difference between the vaccine group and the placebo group was significant (p<0·0001).

Most adverse events were mild. Rates of serious adverse events were similar in the vaccine and placebo groups during the entire follow-up, and none were deemed by the DSMB to relate to vaccination (table 3 and webappendix pp 25–28). Participants in the reactogenicity subset were regularly interviewed by investigators after receipt of each dose to assess adverse events (table 3). In this subset, the proportion of all solicited local adverse events identified within 72 h after each dose was greater in the vaccine group (13·5%) than in the placebo group (7·1%; p<0·0001). The vaccine group also had a greater proportion of adverse reactions attributed to pain, swelling, and itching at injection sites, which were the most common local adverse events. The proportion of systemic adverse events were similar for both groups (20·3% *vs* 19·8%). On the basis of reports by participants not in the reactogenicity subset, the proportion of solicited local adverse events was higher in the vaccine group than in the placebo group (2·8% *vs* 1·9%) and the rates of solicited systemic adverse events were not significantly different between the two groups (table 3).

Serum samples were taken from 11 165 participants before vaccination and 1 month after receipt of the third dose. 5494 (98·7%) of 5567 participants in the vaccine group had an increase in antibody concentration in the samples after vaccination of four times or more from that of the corresponding samples before vaccination. In the samples after vaccination, geometric mean concentration in these participants rose from 0·14 Wu/mL to 19·0 Wu/mL (95% CI 18·6–19·4). By contrast,

	Number of adverse events (rate, 95% CI)		p value*
	Vaccine group	Placebo group	
(Continued from previous page)			
Total vaccinated cohort			
Number of participants who received more than one dose	56302	56302	
Unsolicited events within 30 days after each dose†			
All	6771 (12·0%, 11·76–12·3)	6724 (11·9%, 11·68–12·21)	0·666
≥Grade 3	839 (1·5%, 1·39–1·59)	792 (1·4%, 1·31–1·51)	0·241
Serious adverse events within 30 days after each dose‡			
All	248 (0·4%, 0·39–0·50)	245 (0·4%, 0·38–0·49)	0·892
Admission to hospital	238 (0·4%, 0·37–0·48)	233 (0·4%, 0·36–0·47)	0·817
Disability	0 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	
Death§	10 (0·0%, 0·01–0·03)	12 (0·0%, 0·01–0·04)	0·670
Serious adverse events during period from month 2 to month 6 and from month 7 to month 19†			
All	1423 (2·5%, 2·40–2·66)	1430 (2·5%, 2·41–2·67)	0·894
Admission to hospital	1328 (2·4%, 2·23–2·49)	1336 (2·4%, 2·25–2·50)	0·875
Disability	0 (0·0%, 0·00–0·01)	0 (0·0%, 0·00–0·01)	
Death	95 (0·2%, 0·14–0·21)	94 (0·2%, 0·13–0·20)	0·942

Grade 3 pain, headache, and fatigue were defined as prevention of normal activities; grade 3 swelling was defined as a diameter of more than 30 mm; grade 3 itch was defined as body itch; and grade 3 fever was defined as temperature greater than 39·0°C. Symptoms with frequency more than 1% in any group are listed. The webappendix details all serious adverse events (pp 25–28). *p values are two-sided and were calculated by Fisher's exact test. †Unsolicited adverse events included any adverse events that happened from day 4 to day 30 after each dose and any adverse events within 3 days after each dose but had not been listed in the diary card for registering solicited adverse events. Most often unsolicited adverse events in the study included upper respiratory tract infection, headache, fever, and gastritis. ‡The data and safety monitoring board did not deem any of the serious adverse events to be related to vaccination. §22 participants died within 30 days after each vaccination. Of the ten participants in the vaccine group that died, eight died as the result of an accident, one died of a cerebral haemorrhage, and one died of liver cancer after 10 years with chronic hepatitis B. Of 12 participants in the placebo group that died, six died as the result of an accident, three died of myocardial infarction, two died of cerebral haemorrhage, and one died of stomach cancer.

Table 3: Safety outcomes

119 (2·1%) of 5598 participants in the placebo group showed an antibody response and all the episodes were subclinical infection.

Discussion

In our trial, efficacy of recombinant hepatitis E vaccine during the 12 months from the 31st day after the receipt of the third dose was 100·0% (95% CI 72·1–100·0), and protection was noted across all age and sex subgroups. Vaccination was also beneficial under less than perfect circumstances—ie, when participants did not receive all three doses. Vaccine efficacy after two doses was 100·0% (95% CI 9·1–100·0). Therefore, during a hepatitis E outbreak, or for travellers to an endemic area, protection can be quickly obtained by two vaccine doses given within 1 month.

Side-effects were few and mild and no serious adverse events related to vaccination. HEV 239 is unlikely to induce rare vaccine-related serious adverse events, because the large number of participants in the study affords a power of 85% to detect rare serious adverse events if the rate in the vaccine group is 0·03% and the rate ratio is 5·0 (webappendix p 29).

The study site is endemic for infection with hepatitis E virus, with nearly half the participants tested on day 0 being seropositive. The infection rate in the placebo group was 2·1% during the period from 0 months to 7 months. However, most of the infections seemed to be subclinical and incidence of hepatitis E was estimated to be about four per 10 000 person-years (webappendix p 1). The reason for the low attack rate is unknown. In developed countries where the zoonotic hepatitis E genotype 3 predominates, emerging data showed a moderate hepatitis E virus seroprevalence but rare autochthonous cases of hepatitis E.[3] These findings suggest that the low attack rate might be a common feature of both zoonotic genotypes, relating to a low-level, but nevertheless widespread, exposure in areas where these viruses are prevalent.

Animal studies showed that HEV 239, which is produced with a genotype 1 isolate, confers protection against both genotypes 1 and 4.[19] 12 of 13 patients with hepatitis E who were typed by sequencing, had genotype 4, all in the placebo group. Therefore, our study substantiates that the vaccine cross protects against genotype 4 in human beings, and the cross protection probably extends to other genotypes as well, given that they belong to the same serotype as the vaccine strain.

Data suggest that individuals with chronic liver disease should be prioritised for hepatitis E vaccination to prevent serious damage from infection.[8,9] However, because we excluded this group, additional study is needed to assess the benefits of HEV 239. Another limitation was the lack

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of a hepatitis E case in the vaccine group, meaning that the protective antibody concentration could not be assessed. Further analysis of our serology data might provide important information on the vaccine's efficacy against subclinical infection. Both our study and the previous phase 2 study of the vaccine produced in insect cells showed substantial short-term protection; however, the duration of this protection needs further assessment.

In our trial, we found the vaccine well tolerated and efficacious for a general adult population. Further studies are needed to assess the safety and to support the benefits of the vaccine for pregnant women and for people younger than 15 years or older than 65 years.

Contributors

All authors contributed towards acquisition of data or statistical analyses, or interpretation of data, writing and revising the report, and final approval. F-CZ and JZ contributed equally to this work.

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Conflicts of interest

Y-LX and Y-ML are employees of the Xiamen Innovax. The other authors declare that they have no conflicts of interest.

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18

別紙様式第2

別紙(2)-1

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 2010年11月25日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人 C1-インアクチベーター	研究報告の公表状況	Widespread distribution of hepatitis E virus in plasma fractionation pools Baylis A., Koc O., Nübling C.M., Blümel J.: Paul-Ehrlich-Institut., Langen, Germany Vox Sanguinis 99 (Suppl.1): p320 JUL 2010	公表国 ドイツ	
販売名（企業名）	①ベリナート P ②ベリナート P 静注用 500 （CSL ベーリング株式会社）				

研究報告の概要

背景：E 型肝炎ウイルス（HEV）は、急性肝炎を引き起こす。HEV genotypes 1 および genotypes 2 は、人に限定され、発展途上国での流行と関連しているのに対して、genotypes 3 および genotypes 4 は人畜共通で、発展途上国と先進工業国の両方で人と他のいくつかの動物（特にブタ）を感染させる。
HEV は通常、汚染された水と食物を介して感染する。しかしながら、輸血は、フランス、イギリス、日本を含む数カ国で報告された症例による別の感染経路である。日本と中国の供血者の調査において、HEV RNA は、ALT 値が上昇する場合もしない場合もほぼ 107-108 コピー/mL 程度の高ウイルス量が異なる頻度で検出された。
目的：血漿分画プールの HEV RNA の存在、および、IgG 抗 HEV 抗体の濃度を調べるため。
方法：血漿プールのサンプルは、ORF3 を標的にして一回の反応につき 10 コピーの DNA を検出することができる RT-PCR 法で、HEV RNA の分析がなされた。血漿サンプルは、COBAS AmpliPrep（Roche Diagnostics GmbH、ベンツベルク、ドイツ）と Total Nucleic Acid Isolation kit を用いて抽出された。陽性対照は、HEV 陽性ヒト血漿およびブタサンプルである。
HEV RNA 陽性のプールは、ウエスタンブロッティングおよび ELISA により、IgG 抗 HEV 抗体が調べられた。
結果：約 10%のプールは、HEV RNA 陽性だった。陽性プールの地理的分布は、ヨーロッパ、北アメリカ、東南アジアの数カ国を含む広範囲にわたっていた。プール中で同定された株の系統発生解析により、genotype4 がアジアのプールに限定されていたのに対し、genotype3 はヨーロッパと北アメリカのプールで確認されたことが証明された。
IgG 抗 HEV 抗体と HEV RNA の間で相関関係はみられなかった。抗 HEV の濃度は、全般にアジアのプールでより高かった。
結論：以前 HEV RNA が日本と中国の供血者で確認されたのに対し、血漿分画プールにおける HEV RNA の分析はこれまで報告されたことがない。血漿分画製剤の HEV のリスクを判定するために、更なるプールの HEV RNA 量を測定する調査が現在行われている。

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
血漿分画製剤による HEV 伝播は報告されていない。 仮にHEVが存在する場合にも、液状加熱（パスツリゼーション）による製造工程により低減することから、本剤のHEVに関する安全性は確保できると考える。	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。

(7)

factor VIII injection, the presence of vCJD infectivity in the blood cannot be excluded. This has led to major concerns that a pool of infectious symptomless individuals could exist in the blood donor population leading to further cases of transfusion related person to person disease contamination. The estimated femtomolar sensitivity level required to detect PrP^{TSE} in the plasma of a donor in the pre-clinical phase of infection has been a caveat towards the development of blood screening assays.

Aim: The objective is to develop a sensitive and specific test that enables the confirmation of a PrP^{TSE} positive human blood sample.

Methods: The assay comprises three major steps: (i) selective capture of PrP molecules from human plasma by ligand-coated beads for PrP^{TSE} enrichment and removal of inhibitors present in plasma;(ii) in vitro amplification of PrP^{TSE} by Protein Misfolding Cyclic Amplification (PMCA), in which minute amounts of the captured PrP^{TSE} are amplified in a cyclic process using brain of humanised transgenic mice overexpressing PrP^{MET129} as substrate; (iii) detection by Western Blot of the amplified PrP^{TSE} after proteinase K digestion.

Optimisation of the assay was performed with human plasma samples spiked with serial 10-fold dilutions of 10% vCJD infected brain homogenate.

Results: After capture of PrP^{TSE} from spiked plasma and amplification by three rounds of PMCA, the sensitivity of PrP^{TSE} detection was improved by 5log in comparison with western blot detection only.

Donor	Time to symptoms	Highest temperature	Length of symptoms	Symptoms score	Influenza A (H1N1)PCR
1	4 hours	38.4	2 days	4	Negative
2	30 hours	37.5	4 days	3	Negative
3	12 hours	N.A.	2 days	5	Negative
4	30 hours	39.2	4 days	4	Negative
5	32 hours	38.2	4 days	4	Negative
6	45 hours	N.A.	4 days	3	Negative
7	1 hours	38.5	5 days	5	Negative
8	8 hours	N.A.	3 days	4	Negative
9	48 hours	N.A.	6 days	2	Negative
10	6 hours	38.6	2 days	4	Negative
11	36 hours	N.A.	2 days	4	Negative
12	1 hours	N.A.	3 days	4	Negative
13	27 hours	N.A.	2 days	5	Negative
14	24 hours	38.5	3 days	4	Negative
15	96 hours	40	3 days	6	Negative

Summary/conclusions: We have developed a test that combines a concentration step with the in vitro amplification of PrP^{TSE}, in order to reach the sensitivity required for the detection in blood. The future prospects are: (i) to perform epidemiological studies on at risk population samples from recipients who have received multiple RBC transfusions; (ii) to analyse for confirmation any repeatedly positive result that would be obtained after screening of blood donations by rapid tests.

P-0619

WIDESPREAD DISTRIBUTION OF HEPATITIS E VIRUS IN PLASMA FRACTIONATION POOLS

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Paul-Ehrlich-Institut, Langen, Germany

Background: Hepatitis E virus (HEV) causes acute hepatitis. HEV genotypes 1 and 2 are restricted to humans and associated with epidemics in developing countries, whilst genotypes 3 and 4 are zoonotic and infect humans and several other animals, particularly swine, in both developing and industrialized countries. HEV is normally transmitted via contaminated water and food; however blood transfusion is an alternative route of infection with cases reported in several countries including France, the UK and Japan. In surveys of Japanese and Chinese blood donors, HEV RNA has been detected at varying frequency, both in the presence and absence of elevated levels of ALT, with the highest viral loads in the order of 10^7–10^8 copies/ml.

Aim: To investigate plasma fractionation pools for the presence of HEV RNA and to examine the levels of anti-HEV IgG.

Methods: Samples of plasma pools were analysed for HEV RNA using an RT-PCR method targeting ORF3 and capable of detecting 10 DNA copies per reaction. Plasma samples were extracted using the COBAS AmpliPrep (Roche Diagnostics GmbH, Penzberg, Germany) and the Total Nucleic Acid Isolation kit according to the manufacturer's instructions. Positive controls included HEV positive human plasma and swine samples. Pools positive for HEV RNA were examined for anti-HEV IgG by Western blotting and ELISA.

Results: Approximately 10% of pools were positive for HEV RNA. Positive pools were from a widespread geographic distribution, including several countries in Europe, North America and South East Asia. Phylogenetic analysis of the strains identified in the pools demonstrated that genotype 3 viruses were identified in Europe and North America whilst the genotype 4 viruses were restricted to the Asian pools. No correlation was observed between anti-HEV IgG and HEV RNA. Levels of anti-HEV levels were in general greater in the Asian pools.

Conclusions: Whilst HEV RNA has previously been identified in Japanese and Chinese blood donors, analysis of plasma fractionation pools for HEV RNA has not previously been reported. Further investigations are underway to determine the HEV RNA loads in pools in order to determine the risk of HEV in plasma derived products.

P-0620

HTLV LOOKBACK IN NHS BLOOD AND TRANSPLANT REVEALS THE EFFICACY OF LEUCODEPLETION

Hewitt E[1], Howell D[1], Brant L[2], Taylor G[3]
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Background: Leucodepletion was introduced within NHS Blood and Transplant (NHSBT) in 1998. HTLV screening of blood donations commenced in 2002. The NHSBT national computer database was introduced into blood centres in a phased fashion in the late 1990s. HTLV lookback was performed for previous donations from donors who tested HTLV positive after screening was introduced. Lookback was confined to donations included on the national database.

Aim: Lookback was performed to identify transfusion recipients who had been infected with HTLV through transfusion of cellular blood components from donors who were later identified as HTLV positive.

Methods: Lookback was performed according to established procedures, which were used for the HCV lookback in the 1990s. Previous donations were identified for all HTLV positive donors, and the fate of all cellular components was traced. Recipient hospitals were asked to trace the fate of the components, and to send details of living recipients to NHSBT. The clinician (generally the general practitioner) responsible for the care of the identified recipient was then contacted by the blood service, and notification of the recipient was carried out. Recipients were offered further advice and testing for evidence of HTLV infection. All details were recorded on a database which linked information about donors, donations, blood components and recipients.

Results: Lookback was performed on 413 cellular (red cell and platelet) components issued from 56 blood donors: 83% of components were traced at hospitals to an identified recipient and 182 (53%) of the recipients were known to be dead at the time of reporting. A total of 110 recipients (69% of the living recipients) were tested for HTLV: 80 had received leucodepleted and14 "buffy coat reduced" components, while 16 had received components without any white cell reduction measures. Only six recipients (5.5% of those tested) were positive for HTLV-1. One further recipient was HTLV-2 positive, but all donors were infected with HTLV-1 so this was not judged to be a transfusion-transmission. Overall, infection was present in 5/16 recipients who received non-leucodepleted components compared with 1/80 leucodepleted and 0/14 with buffy coat reduced components. The infected recipients associated with non-leucodepleted components received platelet pools (2) and non-leucodepleted red cells (3) transfused on day 6, 8

別紙様式第2-1

No. 20

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
				2010. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Lo SC, Pripuzova N, Li B, Komaroff AL, Hung GC, Wang R, Alter HJ. Proc Natl Acad Sci U S A. 2010 Sep 7;107(36):15874-9. Epub 2010 Aug 23	米国

研究報告の概要

○慢性疲労症候群(CFS)患者と健康な供血者の血液におけるマウス白血病ウイルス(MLV)関連ウイルス遺伝子配列の検出

CFSは原因不明の重篤な全身疾患である。最近、CFS患者の末梢血に高率に異種指向性マウス白血病ウイルス関連ウイルス(XMRV)由来DNAが検出されたという研究報告があったが、続く4件の研究報告は、それを証明できていない。

今回、CFS診断基準を満たす患者37名からの末梢血単核細胞由来DNA 41検体を調べ、37名中32名にMLV関連ウイルスgag遺伝子配列を認めたが、健常ボランティア供血者では44名中3名にしか認められなかった。PCR分析システムまたは臨床検体において、マウスDNA混入の証拠は検出されなかった。およそ15年後に得られた検体において、gag陽性患者8名中7名が再度陽性となった。全てのXMRVが遺伝的に近縁という報告知見に反して、遺伝的に多様なグループのMLV関連ウイルスを確認した。CFS患者に由来するgagおよびenv 配列は、XMRVよりも多指向性マウス内在性レトロウイルスに近縁であり、さらに、同種指向性のMLV配列とは近縁性が低かった。MLV関連ウイルスと同一の強い関連性があるかどうか、これらウイルスがCFS発症において原因的役割を担っているかどうか、ならびにこれらが血液供給の脅威となるかについては、さらなる研究が必要である。

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
慢性疲労症候群患者37名の末梢血単核細胞由来DNAを調べたところ、32名にマウス白血病ウイルス関連ウイルスgag遺伝子配列を認めたが、健常者ボランティアでは44名中3名にしか認められなかったとの報告である。	日本赤十字社では、献血の間診時に献血者の健康状態を把握・確認している。平成22年5月18日に開催された平成22年度第1回血液事業部会運営委員会において、XMRVとCFSとの関連について、現時点で緊急的な対応をとる必要はないものの、引き続き情報収集を行い、新たな知見等が得られれば、本委員会において対応を検討することとされた。今後も引き続き、情報の収集に努める。

3

Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors

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Contributed by Harvey J. Alter, May 25, 2010 (sent for review March 23, 2010)

Chronic fatigue syndrome (CFS) is a serious systemic illness of unknown cause. A recent study identified DNA from a xenotropic murine leukemia virus-related virus (XMRV) in peripheral blood mononuclear cells (PBMCs) from 68 of 101 patients (67%) by nested PCR, as compared with 8 of 218 (3.7%) healthy controls. However, four subsequent reports failed to detect any murine leukemia virus (MLV)-related virus gene sequences in blood of CFS patients. We examined 41 PBMC-derived DNA samples from 37 patients meeting accepted diagnostic criteria for CFS and found MLV-like virus *gag* gene sequences in 32 of 37 (86.5%) compared with only 3 of 44 (6.8%) healthy volunteer blood donors. No evidence of mouse DNA contamination was detected in the PCR assay system or the clinical samples. Seven of 8 *gag*-positive patients tested again positive in a sample obtained nearly 15 y later. In contrast to the reported findings of near-genetic identity of all XMRVs, we identified a genetically diverse group of MLV-related viruses. The *gag* and *env* sequences from CFS patients were more closely related to those of polytropic mouse endogenous retroviruses than to those of XMRVs and were even less closely related to those of ecotropic MLVs. Further studies are needed to determine whether the same strong association with MLV-related viruses is found in other groups of patients with CFS, whether these viruses play a causative role in the development of CFS, and whether they represent a threat to the blood supply.

xenotropic murine leukemia virus-related virus | murine leukemia virus-like virus | viral gag gene sequence | polytropic | mouse mitochondria DNA PCR

Chronic fatigue syndrome (CFS) is a debilitating disorder defined solely by clinical symptoms (1) and the exclusion of other diseases; its distribution is wide and its cause is unknown. In many instances, the illness starts suddenly with an infectious-like syndrome. A number of objective immunological and neurological abnormalities have been found more often in patients with CFS than in healthy controls or in patients with other fatigue-inducing illnesses (2). Various microbial and viral infections have been implicated as possible triggers of CFS, including human herpesvirus-6, Epstein–Barr virus, enteroviruses, parvovirus B19, and the bacteria that cause Lyme disease and Q fever (2). However, no single agent has been associated with a large fraction of cases.

A recent study reported that a high percentage of patients with CFS are infected with a mouse leukemia retrovirus that has been designated xenotropic murine leukemia virus-related virus (XMRV) (3), a virus first identified in samples of human prostate cancer tissue about 4 y ago (4). However, two subsequent studies failed to find an infectious murine leukemia virus (MLV)-related virus in German prostate cancer patients (5, 6), and four recent studies from Europe and the United States have failed to detect XMRV or an MLV-related viral gene sequence in the blood of CFS patients using PCR (7–10).

In the mid-1990s, we obtained serum and whole-blood samples from CFS patients for the investigation of possible mycoplasmal infections (11). Whole-blood, peripheral blood mononuclear cell (PBMC), and plasma samples from 37 CFS patients in the mycoplasma studies were maintained in frozen storage at −80 °C. Twenty-five patients were from an academic medical center and 12 were referred by community physicians. Repeat blood samples were obtained from the academic medical center patients: four samples were obtained 2 y later and similarly kept in frozen storage, eight were obtained ~15 y later, in 2010, and processed for XMRV/MLV-related virus testing without being frozen.

By nested PCR assays targeting the MLV-related virus *gag* gene, using both the previously described primer sets (3, 4) and an in-house–designed primer set with highly conserved sequences from different MLV-like viruses and XMRVs, we examined DNA prepared from the blood samples of these 37 CFS patients for the presence of MLV-like virus *gag* gene sequences. In addition, RNA was prepared from the deep-frozen plasma samples of these patients and analyzed by RT–PCR assay. DNA extracted from frozen PBMC samples of 44 healthy volunteer blood donors was tested in parallel.

Results

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of CFS Patients. By nested PCR assays, targeting the mouse retrovirus *gag* gene using either the previously reported PCR primer sets (first round: 419F/1154R; second round: GAG-I-F/GAG-I-R) (3, 4) or our in-house–designed PCR primer set (first round: 419F/1154R; second round: NP116/NP117) (Fig. 1), we detected a high frequency of MLV-related virus *gag* gene sequences in patients with CFS. The NP116/NP117 is an internal primer set with highly conserved sequences in different MLV-like viruses and XMRVs (Fig. S1). After the first round of nested PCR using primer set 419F/1154R, gel electrophoresis revealed positive PCR-amplified products with the predicted size of ~730 bp in 21 of 41 PBMC or whole-blood samples from 37 CFS patients (Fig. 1*A*). The nested PCR results produced by the second round of amplification using either the internal primer set GAG-I-F/GAG-I-R (with a predicted size of an ~410-bp product) or the internal primer set NP116/NP117 (with a predicted size of an ~380-bp product) were essentially identical. Overall, samples from 32 of 37 (86.5%) CFS patients revealed positive amplification products with the correct predicted sizes in the nested PCR (Fig. 1*B*). Of the 25 CFS patients who had been rigorously evaluated at the academic

Author contributions: S.-C.L., N.P., and B.L. designed research; G.-C.H. designed mouse-specific mitochondria PCR assay; N.P. and B.L. performed research; B.L. and R.W. contributed new reagents/analytic tools; S.-C.L., N.P., G.-C.H., and R.W. analyzed data; and S.-C.L., N.P., A.L.K., and H.J.A. wrote the paper.

The authors declare no conflict of interest.

See Commentary on page 15666.

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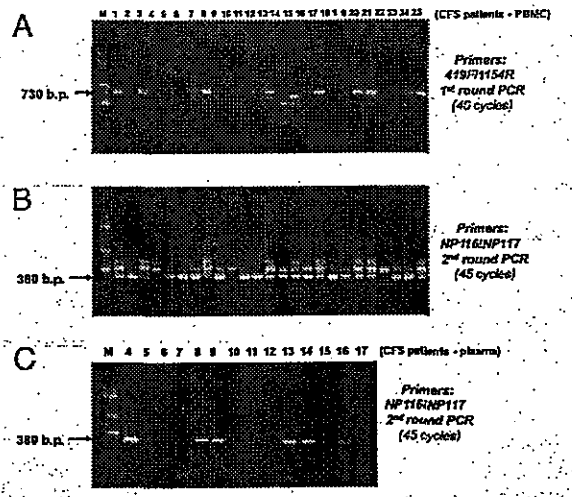

Fig. 1. MLV-related *gag* gene sequences detected in blood DNA from CFS patients. (*A*) Results of PBMC DNA from CFS patient samples 1–25 (of 41 samples examined) amplified after the first round of nested PCR using a previously published primer set (419F/1154R): targeting *gag* gene. (*B*) Results of PBMC DNA from the 25 CFS samples after completing the second round of nested PCR using an in-house–designed PCR primer set (NP116/NP117). (*C*) MLV-related *gag* gene RNA sequences are detected in plasma of CFS samples by RT-nested PCR. Results of RT-nested PCR for RNA derived from the plasma samples of CFS patients 4–17 are shown. The positions of expected sizes of the "positive" PCR amplicons are indicated by arrows. M, DNA ladder size markers. All positive PCR amplicons with the expected size have been confirmed by DNA sequencing.

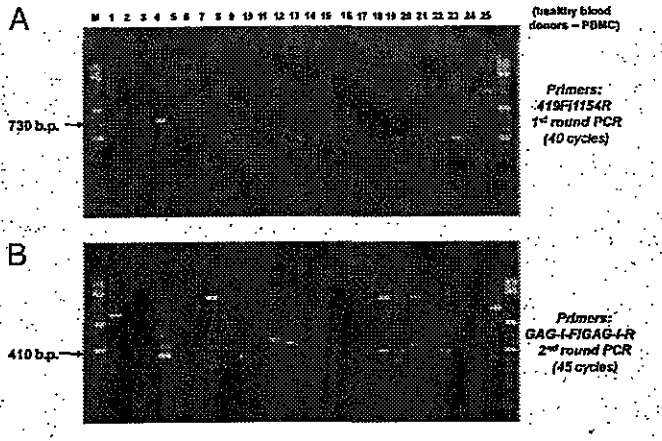

Fig. 2. MLV-related *gag* gene sequences detected in normal blood donors by nested PCR. (*A*) Results of PBMC DNA from blood donors 1–25 (of 44 donors examined) amplified after the first round of nested PCR using primer set 419F/1154R. Lane 4: PBMC DNA from BD22 has a positive target PCR amplicon confirmed by sequencing. (*B*) Results of PBMC DNA from the 25 normal blood donors after the second round of nested PCR using PCR primer set GAG-I-F/GAG-I-R (4). Sequencing of the PCR bands with size ~413 bp revealed that lane 4 (BD22), lane 7 (BD26), and lane 9 (BD28) were MLV-like virus *gag* gene sequences; lane 8 (BD27) was a human sequence. The positions of expected sizes of the positive PCR amplicons are indicated by arrows. M, DNA ladder size markers.

medical center, 24 (96%) were positive. On repeated testing 2 y later of four of the academic center patients, all four remained positive. On repeated testing of eight academic center patients ~15 y later (in 2010), seven remained positive. All PCR products with the correct predicted size were retrieved from the gel and analyzed by DNA sequencing. Their DNA sequences were all confirmed to be those of MLV-related virus *gag* genes. The alignments of all of the sequences obtained from PCR products of ~730 bp are shown in Fig. S1. All of the positive PCR products amplified from the CFS patients' samples using primer set 419F/1154R were 746 bp in length. All of the positive PCR products amplified from the patients' blood samples using primer set GAG-I-F/GAG-I-R and primer set NP116/NP117 were 413 and 380 bp in length, respectively.

In 42% of samples, we also detected and sequence-confirmed the presence of MLV-related viral RNA in the frozen plasma samples of these CFS patients, using an RT–PCR assay (Fig. 1*C*). With one exception, all of the patients who tested positive for viral RNA *gag* gene sequences in the plasma samples also tested positive in the DNA prepared from PBMCs and/or whole blood. On the other hand, only about half of the patients with MLV-related virus *gag* gene sequences detected in PBMC DNA also had viral *gag* RNA sequences detected in the plasma.

MLV-Related Viral *gag* Gene Sequences Detected in the Blood of Healthy Volunteer Blood Donors. DNA originating from 44 healthy volunteer blood donors was tested in parallel by nested PCR (Fig. 2). The nested PCR testing using the MLV-related virus *gag* gene-specific primer sets could produce many side products from human DNA (Figs. 1 and 2). We sequenced all of the PCR-amplified DNA bands from the 44 control samples of blood donors having molecular sizes close to that of the predicted PCR products from the target XMRV *gag* gene in the first round of PCR (Fig. 2*A*) and in the second round of nested PCR (Fig. 2*B*). After the first round of amplification in nested PCR, a positive PCR product with the predicted size of ~730 bp was found in PBMC DNA from 1 of 44 blood donor controls (lane 4, Fig. 2*A*). This ~730-bp PCR product amplified from the blood donor (BD22) was confirmed by DNA sequencing as an MLV-related virus *gag* gene sequence of 745 bp (Fig. S1). Overall, we found 3 of 44 (6.8%) blood donors' PBMCs (BD22, BD26, and BD28) to be positive for the MLV-related virus *gag* gene sequences by completing both rounds of nested PCR (Fig. 2*B* and Fig. S2).

MLV-Related Viral *env* Gene Sequences Detected in the Blood of a CFS Patient and a Healthy Blood Donor. PBMC DNA from all of the CFS patients and healthy blood donors was also tested by PCR, targeting various regions of the MLV-related viral *env* gene. The MLV-related viral *env* gene segment of 240 bp was amplified and confirmed by sequencing from one healthy donor (BD-26) by a semi-nested PCR using the primer set 5922F/6273R in the first round of amplification and 5922F /6173R in the second round of amplification. The MLV-related viral *env* gene segment of 206 bp was amplified and confirmed by sequencing from 1 CFS patient by a nested PCR using primer set 5922F/6273R in the first round of amplification and 5942F/6159R in the second round of amplification (*SI Materials and Methods*).

Phylogenetic Analyses of MLV-Related Virus *gag* and *env* Gene Sequences. Multiple sequence alignment (MSA) and phylogenetic analysis of the MLV-related virus *gag* gene sequences amplified from 21 CFS patient samples and one blood donor (BD22) are shown in Fig. S1 and Fig. 3*A*, respectively. There were three different MLV-related retroviral *gag* gene sequences identified by PCR in the blood samples of the CFS patients and a fourth variant was detected in blood donor BD22. The sequences in all four variants were more closely related to the sequences of polytropic mouse endogenous retroviruses (mERVs) than to those of XMRVs. Although variations were observed, the majority (18/21, 86%) of CFS patient samples had the same viral *gag* gene sequence (CFS type 1), whereas 2/21 had a different, but similar, viral *gag* gene sequence (CFS type 2), and a third distinct sequence (CFS type 3) was found in the remaining CFS case. Phylogenetic analysis using the 746-nt sequences obtained revealed that CFS type 1, CFS type 2, and CFS type 3 formed a cluster that is clearly separable from the cluster formed by the newly reported XMRVs (Fig. 3*A*). Interestingly, the 745-nt virus *gag* gene sequence of donor BD22 (with a 1-nt deletion in the alignment) could not be included in

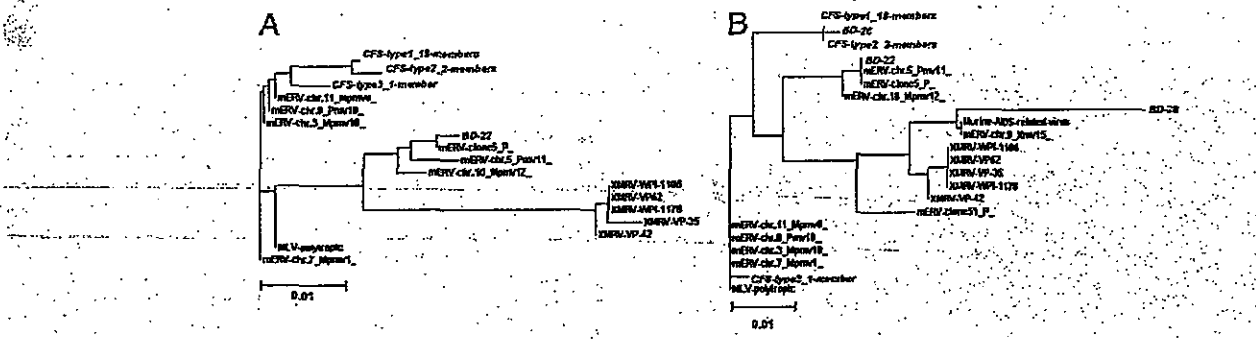

Fig. 3. Phylogenetic trees corresponding to the MSAs shown in Figs. S1 and S2 were generated by the ClustalW2 program using the neighbor-joining method (*Materials and Methods*). (*A*) Phylogenetic analysis based on the 746-nt *gag* gene nucleotide sequences amplified from blood samples of CFS patients and BD-22 of the corresponding MSA in Fig. S1. (*B*) Phylogenetic analysis based on the 380-nt *gag* gene sequences amplified from blood samples of CFS patients and healthy blood donors using the primer set NP116/NP117 of the corresponding MSA in Fig. S2.

either the cluster of CFS type 1/CFS type 2 or the cluster of XMRVs. The viral *gag* gene sequences of CFS type 3 and blood donor BD22 appear to be phylogenetically more closely related to polytropic mERVs or modified polytropic mERVs (Fig. 3*A*).

Sequence analysis of the shorter fragments of the viral *gag* gene amplified from blood of 36 out of 41 CFS patient samples and 3 out of 44 blood donor samples after the second round of nested PCR similarly confirms that there are significant variations among the MLV-like *gag* gene sequences. Fig. S2 shows sequence alignment of the 380-nt segments of viral *gag* genes amplified from three blood donors (BD22, BD26, and BD28), patients with CFS types 1–3, XMRVs, and other closely related mERVs/polytropic MLVs. As an example, the viral *gag* gene sequence identified in BD28, but not sequences of BD22 and BD26, has a prominent 21-nt deletion that is uniquely present in polytropic mERV clone 51 (Fig. S2). In phylogenetic analysis, the 380-nt segment of the *gag* gene sequence found in BD26 appears to be closely related to those of CFS types 1 and 2. However, similar to the analytic result with the 746-nt product, the 380-nt *gag* gene sequences of BD22 and BD28 again cannot be included in either the cluster of CFS type 1/CFS type 2 or the cluster of XMRVs (Fig. 3*B*). On the other hand, when the protein sequences coded by the *gag* gene sequences identified in the CFS patients and blood donors are compared with those of a wider range of exogenous and endogenous MLVs, they are most similar to those of polytropic MLVs and XMRVs (Fig. S3). A ClustalW Gag protein tree again reveals that MLV-like virus *gag* proteins have much more similarity to those of modified-polytropic and polytropic mERVs or to those of XMRVs, but are very different from those of exogenous ecotropic MLVs (Fig. 4).

The sequence alignment and the phylogenetic analysis of the MLV-related virus *env* gene sequences obtained from both the CFS patient and healthy blood donor revealed that they were also more closely related to those of polytropic or modified polytropic MLVs than to those of XMRVs (Fig. S4).

Testing for the Presence of Mouse DNA in Patient and Blood Donor Samples Positive for MLV-Like *gag* Gene Sequences. Mouse DNA contains endogenously many closely related proviruses of MLVs. Hence, contamination of the blood samples or reagents by mouse DNA could have produced falsely positive PCR results. Although we took great precautions to prevent potential contamination in the laboratory, and although multiple negative controls were always included in each assay, we took additional steps to confirm that no mouse DNA had contaminated the assays or the clinical samples prepared in this study. We estimated that there were about 200–1,800 mitochondrial DNA (mtDNA) copies per mammalian cell. A highly sensitive PCR assay targeting mouse-specific mtDNA was developed (*Materials and Methods*) to exclude any possible minute mouse DNA contamination in the assay system and in the clinical samples with positive amplified *gag* gene products.

The first round of the semi-nested PCR (40 cycles) used primer set mt15982F/mt16267R and could detect 10 fg of mouse DNA in the presence of 35 ng of human background DNA. By comparison, when studied in parallel under the same assay conditions, the first round of nested PCR (40 cycles) against the MLV *gag* gene required ~10 pg of mouse DNA to detect viral gag gene sequences (Fig. 5*A*). Thus, the first round of mouse-specific mtDNA PCR assay could detect a positive mtDNA signal at a mouse DNA concentration 1,000-fold below the concentration required to detect a positive *gag* signal. The second round of mouse mtDNA semi-nested PCR, using primer set mt16115F/mt16267R, could consistently amplify the target 153-bp amplicon from 2.5 fg of mouse DNA mixed with 35 ng of human DNA (Fig. 5*B*). By comparison, the second round of the nested *gag* gene PCR produced positive ~400-bp amplicons from 500 fg of mouse DNA mixed with 35 ng of human background DNA in the reaction. Thus, the second round of mouse mtDNA semi-nested PCR had a sensitivity hundreds-fold higher than that of the second round of the MLV *gag* gene nested PCR in amplifying mouse DNA.

Using this highly sensitive PCR assay for mouse-specific mtDNA, we examined all of the blood samples that were found positive for MLV-like virus *gag* gene sequences from both CFS patients and healthy controls for evidence of mouse DNA contamination. PBMC DNA (30–40 ng) from the CFS patients

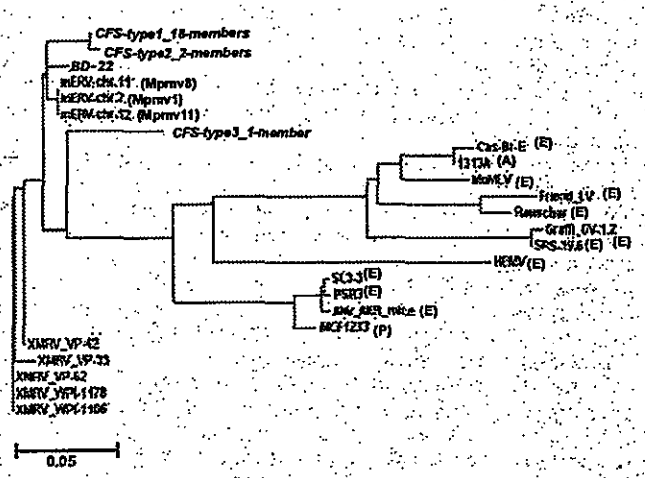

Fig. 4. Phylogenetic analysis of protein sequences based on the alignment shown in Fig. S3. CFS types 1, 2, and 3 and BD-22 and MLVs *gag* protein sequences are compared. Gag protein sequences starting from the AUG initiation codon are aligned with those of relevant endogenous as well as exogenous MLVs. Sequences of MLVs are referred to as polytropic (P), ecotropic (E), amphotropic (A), or modified polytropic (Mpmv). MoMLV, Moloney murine leukemia virus; HEMV, hortulanus endogenous murine virus.

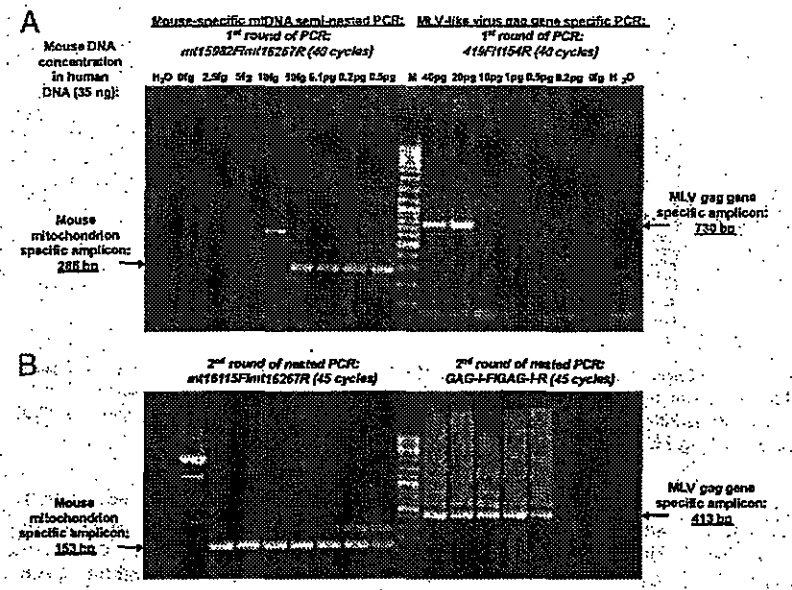

Fig. 5. Comparison of sensitivity in amplifying mouse DNA by the semi-nested PCR targeting mouse-specific mtDNA and by the nested PCR targeting MLV-like virus *gag* gene. Serial dilutions of mouse spleen DNA (from 40 pg to 2.5 fg) were spiked into 35 ng of total human PBMC DNA and compared in parallel for the mouse DNA detection sensitivity of the two PCR assays. (*A*) In the first round of the mtDNA-specific PCR assay, 10 fg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the 286-bp target product. In the first round of MLV *gag* gene nested PCR assay, 10 pg or more of mouse DNA could be detected in the presence of 35 ng of human DNA by producing the ~730-bp target product. (*B*) In the second round of mouse-specific mtDNA semi-nested PCR, the 153-bp target amplicon could consistently be amplified from 2.5 fg of mouse DNA. In the second round of *gag* gene-specific nested PCR, the 413-bp target product could be amplified from 0.5 pg or more of mouse DNA. Lane 0 fg: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix. Primers and PCR cycle numbers used in each round of amplification for both of the assays are shown at the top of each gel.

and the healthy blood donors, as well as serial dilutions from 50 to 1 fg of mouse DNA mixed with 35 ng of human DNA as the positive templates, were tested in parallel. No mouse DNA was found in the PCR mix nor in the blood samples of CFS patients and blood donors that tested positive for the MLV-like virus *gag* gene sequences. Fig. 6 shows the results of the two rounds of mouse-specific mtDNA semi-nested PCR testing in DNA from PBMCs of four CFS patients (patients 8, 17, 20, and 25) with positive 746-bp amplicons in the first round of the nested PCR targeting the MLV-like virus *gag* gene, as well as from three blood donors (BD22, BD26, and BD28) who tested positive and two donors (BD21 and BD23) who tested negative for MLV-like *gag* gene sequences.

Discussion

Detection of MLV-Related Nucleic Acid Sequences. Our laboratory detected MLV-related virus *gag* gene sequences in DNA from PBMC and whole-blood samples from 32 of 37 (86.5%) CFS patients, compared with 3 of 44 (6.8%) volunteer blood donors, using a two-round nested PCR. Following only one round of PCR amplification, 21 of the 41 CFS patients' DNA samples were found positive compared with only 1 of 44 donor samples. In every instance throughout these studies, the "positive" result by PCR (an amplicon of the predicted size) was confirmed by sequencing.

In four CFS patients from whom two samples were obtained, 2 y apart, the *gag* gene sequences were detected on both occasions. Further, *gag* gene sequences were still detectable in seven of eight CFS patients from whom fresh samples were obtained ~15 y after they were initially found to be MLV *gag* gene positive. In one *gag*-positive CFS patient and one *gag*-positive blood donor, MLV-related *env* gene sequences also were detected by PCR. However, we were unable to PCR amplify and determine the MLV-related *env* gene sequences in the majority CFS patients, possibly because of the low copy number and the greater genetic variability in the *env* gene compared with the *gag* gene.

In the CFS patients, plasma samples revealed MLV-related virus *gag* gene sequences in 42% when tested by RT–PCR for viral RNA. Whereas all but one patient whose plasma tested positive for viral RNA also tested positive in PBMCs for viral DNA, only half of the cases in which MLV-related virus *gag* gene sequences were detected in PBMCs had detectable RNA sequences in plasma. Thus, accurate determination of the prevalence of these agents in patients and donors requires cellular DNA for analysis.

Sequence Variability. Previous reports of XMRV isolates from patients with CFS and with prostate cancer and from individuals in different geographic locations have described very similar nucleic acid sequences (3, 4, 12), a feature believed to be a unique characteristic of XMRVs (13). However, our analysis revealed three different types of MLV-related virus *gag* gene sequences in CFS patients. In all three groups, the sequences were more closely related to the sequences of polytropic mERVs than to XMRVs and were more distant from the sequences of ecotropic MLVs (Fig. 3). Moreover, viral *gag* gene sequences with significant variations from both the cluster of CFS type 1/CFS type 2 and the cluster of XMRVs were identified in at least two blood donors (BD22 and BD28); phylogenetic analysis revealed the latter sequences to be more closely related to those of polytropic or xenotropic mERVs (Fig. 3*B*). It is unclear whether the sequence variations of the viral genes identified in the CFS patients and healthy blood donors have any significance in viral pathogenesis or disease development.

The MLV-like virus *gag* gene sequences in the CFS patients and blood donors had a deletion of 9 nt in the 5′ *gag* leader region and did not have the 24-nt deletion in this region reported in XMRVs. Internal deletions of 9 nt similar to what we have identified in the CFS patients and the blood donors are known to be present in the region that encodes the glycosylated Gag protein (GlycoGag) in some infectious endogenous (ecotropic) MLVs and exogenous (xenotropic) MLVs, such as AKV and DG-75 (14, 15). Many previous studies have shown that the nonstructural GlycoGag of MLVs plays a critical role in viral pathogenesis and in vivo infectivity (16–19). In this context, the MLV-like virus *gag* gene sequences identified in most of our CFS patients (both CFS type 1 and CFS type 2) appear to have an intact GlycoGag in-frame with the matrix and are consistent with the gene sequences of infectious MLVs. Unfortunately, the sequences presently obtained in the study are still a bit short and lack the alternative start codon CUG. Slight extension of the 5′ leader sequence will be needed to confirm the intact nature of GlycoGag.

Could Our PCR Results Have Been Falsely Positive? Voisset and coauthors (20) recently reviewed the pitfalls encountered in the identification of new retroviruses ("rumor viruses"). False-positive results can occur for a variety of reasons. Viral gene sequence-specific PCR primers can nonspecifically amplify nucleic acid sequences that differ from the target sequence. For this reason, we sequenced every positive PCR product (every amplicon of the predicted size) and confirmed MLV-related gene sequences in every instance.

Although contamination must always be a concern for any PCR-based study, several pieces of evidence argue against the

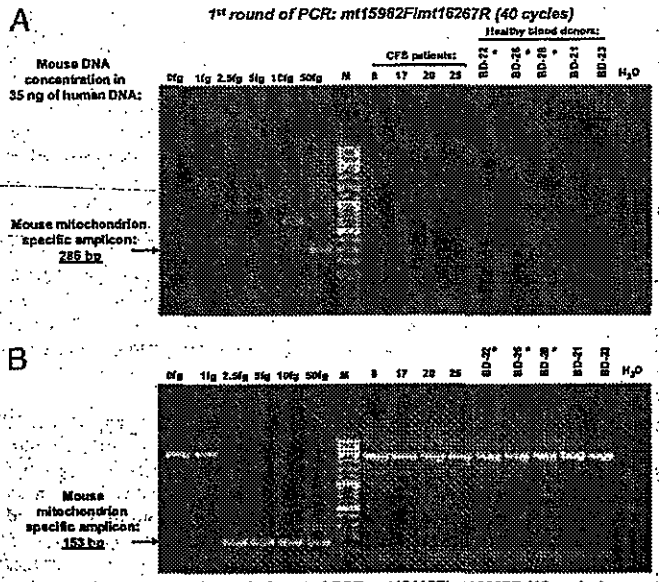

Fig. 6. Testing of CFS patients' and healthy blood donors' samples positive for MLV-like *gag* gene sequences for the presence of mouse DNA contamination using the semi-nested PCR assay targeting mouse-specific mtDNA. Serial dilutions of mouse DNA were spiked into 35 ng of human DNA and used as the controls of the assay sensitivity. The first round of mouse mtDNA semi-nested PCR (*A*) detected 10 fg of mouse DNA, and the second round of the semi-nested PCR (*B*) detected 2.5 fg of mouse DNA in the presence of 35 ng human background DNA. No evidence of mouse DNA contamination could be found by either round of mouse mtDNA semi-nested PCR in the PBMC DNA (35 ng) of CFS patients (patients 8, 17, 20, and 25); three blood donors (BD-22, BD-26, BD-28) tested positive and two blood donors (BD-21 and BD-23) tested negative for the MLV-like virus *gag* gene sequences. Healthy blood donors' samples positive for MLV-like *gag* gene sequences are labeled by asterisks. Lane 0: 35 ng of human DNA without spiking any mouse DNA. Lane H_2O: No DNA template. M: 100-bp DNA ladder mix.

possibility that the PCR products identified in our study are the result of laboratory contamination. First, every clinical sample that tested positive for the MLV-like virus *gag* gene sequences was tested for evidence of mouse DNA contamination using a semi-nested PCR for mouse-specific mtDNA that was exponentially more sensitive in detecting mouse mtDNA than MLV-related *gag* sequences (Fig. 5). Any detection of MLV *gag* that was caused by contamination with mouse DNA also would have detected mouse mtDNA by PCR, thereby identifying the *gag* result as falsely positive. In fact, no positive signal was detected by the mtDNA semi-nested PCR assay in any of the reaction mixtures or in the DNA of clinical samples examined in the study, thus excluding possible contamination by mouse DNA.

Second, we addressed the possibility that the clinical samples or the assay system might have been contaminated. The blood samples were obtained in clinical laboratories that never worked with mice or retroviral vectors and were drawn through sterile needles into vacuum tubes that remained unopened until testing. The laboratory in which PCR testing was performed also had never worked with murine cells, tissues or serum samples, or MLV vectors. Finally, because repeated entry into samples would increase the chance of contamination, we emphasize that sample vials from both patients and normal donors had never been entered before our testing.

Third, there were at least six different MLV-related *gag* gene sequences amplified from the blood samples of CFS patients and blood donors. Typically, contamination would be manifest as the same sequence in all or most samples. Moreover, the sequences that we observed all had significant variations from the previously reported exogenous MLVs or viral vectors. Furthermore, during this study more than 300 negative controls set up for multiple PCR amplification assays targeting the MLV *gag* gene were performed, and all were negative.

Finally, a new set of blood samples was recently obtained from 8 of the original 25 patients followed in an academic medical center. Testing of the repeat blood samples, ~15 y after the index sample, showed that seven of eight patients examined had detectable MLV-*gag* gene sequences. Significant variations of MLV-like virus *gag* gene sequences amplified from the freshly obtained blood samples were identified as would be expected in retroviral infections, but not from contamination.

The ultimate proof of low-grade infection by MLV-related viruses in humans may rely on demonstrating the integration of the viral genes into the human genome (20). The identification of provirus integration sites will take more time and effort to investigate, given that we estimate only one virus gene copy in every 400–4,000 nucleated PBMCs. Also, previous work with XMRV indicates that integration sites are quite variable (21) and the same may be true for the polytropic mouse endogenous retroviruses, which are predominant in this study.

Why Have Other Studies Come to Different Conclusions? Although we find evidence of a broader group of MLV-related viruses, rather than just XMRV, in patients with CFS and healthy blood donors, our results clearly support the central argument by Lombardi et al. (3) that MLV-related viruses are associated with CFS and are present in some blood donors. However, four recent studies have failed to confirm the PCR results reported by Lombardi (7–10). There are various possible explanations for this disparity. As stated in the reports, there could be a difference in the prevalence of these infectious agents in CFS patient populations in different geographic areas. This argument is somewhat less plausible since the publication of a recent negative study with subjects from the United States (10). Nevertheless, the heterogeneity in *gag* gene sequences that we observed suggests that geographic differences in different MLV-related viruses may be considerable and could affect both the sensitivity and the specificity of molecular amplification using standard primer sets.

Indeed, it is possible that the PCR primers used in various studies may have different sensitivity in detecting the diverse group of MLV-related virus *gag* gene sequences that we found in the clinical samples. The 5′ *gag* leader sequence of previously described XMRVs represents the most divergent segment of the XMRV genome in comparison with the genomes of the other MLVs (4). In particular, there is evidently a unique 15-nt deletion in the 5′ *gag* leader region in all of the XMRVs previously identified in patients with prostate cancer and CFS (3, 4). To detect XMRVs in human samples with better sensitivity and specificity, some studies used a PCR primer spanning this unique deletion as the "XMRV-specific" primer (6). However, none of the viral *gag* gene sequences amplified from the blood samples of CFS patients and blood donors in our study has this particular deletion (Fig. S1). As a consequence, such primers might have been insensitive in detecting the MLV-related *gag* gene sequences that we have identified.

Finally, it is also quite possible that there is heterogeneity in the patients diagnosed with CFS in different studies. CFS is a syndrome defined exclusively by a group of nonspecific symptoms and thus has an ill-defined phenotype. Future studies should adhere to consensus case definitions such as that developed by the Centers for Disease Control and Prevention (CDC) (1). Conversely, putative "healthy" control subjects should explicitly deny the presence of those symptoms that constitute the case definition of CFS. Furthermore, even bona fide cases of CFS may have different viral or other etiologies.

Further Considerations. The finding of XMRV or MLV sequences in persons with CFS or other diseases does not constitute definitive proof of viral infection. However, in the study of Lombardi et al. (3) and studies reviewed subsequently by Silverman et al. (22) the evidence for XMRV infection in humans not only involved detection of viral nucleic acids using PCR, but also reported the detection of

viral antigens, detection of anti-viral antibodies, the ability to culture the virus in a prostate cancer cell line, the detection of gamma retrovirus particles by electron microscopy, and transmission of infection to macaques. In sum, none of the four studies that have failed to confirm the PCR evidence reported by Lombardi et al. (3), nor our own study, has attempted to fully replicate that study.

It remains to be shown that the association that we have found, using the methods that we have described, can be generalized to a larger group of patients with CFS. Indeed, we suspect that the association will be lower in CFS cases identified through community-based surveys, as contrasted to cases seen at academic medical centers. Even if subsequent studies confirm an association between MLV-like viruses and CFS, that will not establish a causal role for these viruses in the pathogenesis of this illness. For example, such a high frequency of infections with MLV-related viruses in patients with CFS could reflect an increased susceptibility to viral infections due to an underlying CFS-related immune dysfunction, rather than a primary role for these viruses in the pathogenesis of CFS.

Finally, the finding of MLV-related virus *gag* gene sequences in nearly 7% of healthy volunteer blood donors in our study and of XMRV in 3.7% of healthy controls in the study of Lombardi et al. (3) raises additional issues. The possibility that these agents might be blood-transmitted and pathogenic in blood recipients warrants extensive research investigations of appropriately linked donor–recipient cohorts.

Materials and Methods

Samples from CFS Patients and Healthy Controls. Initially, we tested 41 whole-blood samples that had been obtained for culture isolation of mycoplasmal agents in the mid-1990s. We maintained whole-blood, PBMC, and plasma samples from CFS patients in frozen storage at −80 °C. Of the 41 patient samples, 29 were collected from 25 patients by one of us (A.L.K.) at the Chronic Fatigue Research Center, Brigham and Women's Hospital (Boston, MA). Four of the CFS patients also had blood obtained on a second occasion ~2 y later. Most of the patients were from the New England area; none were related, and virtually none had any regular social contact. Each of the 25 patients was systematically evaluated with a standardized history (supplemented by a patient questionnaire), physical examination, and battery of laboratory tests. Each met the 1988 CDC criteria for CFS, and 21 also met the 1994 CDC criteria. The average age of the patients at the time of venepuncture was 44.4 y; 4 were male and 21 were female. All of the patients signed informed consent documents approved by the Institutional Review Board of Brigham and Women's Hospital. A new set of blood samples was obtained in 2010 from 8 of the original 25 patients followed in the academic medical center. The blood samples were processed for PCR study without first being frozen. The other 12 samples from CFS patients were sent by individual clinicians taking care of patients in the mid-1990s who were given the diagnosis of CFS. We do not have details regarding the methodology by which the referring clinicians established the diagnosis of CFS. The samples had also been sent in the mid-1990s and stored at −80 °C. Frozen PBMC samples from 44 normal blood donors from the Washington, DC, area were collected in 2003–2006 and stored at the Department of Transfusion Medicine, Clinical Center, National Institutes of Health. All patient and control samples were coded and tested in parallel. Details of the preparation of blood samples and DNA/RNA isolation are described in *SI Materials and Methods*.

XMRV/MLV *gag* Nested PCR. The nested PCR for the *gag* gene was performed according to the protocols described previously (3, 4) with minor modifications. Three primer sets used in the study are as follows: 419F and 1154R (3), GAG-I-F and GAG-I-R (4), NP116 forward, and NP117 reverse. The NP116/NP117 was an in-house–designed primer set based on the highly conserved sequences found in different MLV-like viruses and XMRVs (Fig. S1). Primer sequences and details of PCR sensitivity and specificity quality controls can be found in *SI Materials and Methods*.

Phylogenetic Analysis. To generate the neighbor-joining phylogenetic tree, the viral *gag* gene sequences obtained from blood samples of patients with CFS, normal blood donors, as well as all of the closely related MLV *gag* gene sequences selected from the National Center for Biotechnology Information (NCBI) database by BLAST querying with the *gag* gene sequences obtained in the PCR study (*SI Materials and Methods*) were aligned with ClustalW2 (http://www.ebi.ac.uk/tools/clustalw2) using default settings. The analysis produced the same phylogenetic trees with or without consideration of the sequence gaps in alignment.

Mouse Mitochondrial DNA Assay. The complete mtDNA sequences of humans and mice were downloaded from GenBank and aligned using ClustalW. Sequence alignment revealed the 439 bp of the 3′ end of mouse mtDNA (beyond 15,862 bp, according to the coordinates of BALB/c mouse; accession no. AJ512208) were not present in human mtDNA. Primer sets were designed for a semi-nested, mouse-specific mtDNA PCR based on the sequence in this region of mouse mtDNA using Primer-Blast from NCBI. The external PCR primers (*SI Materials and Methods*) were designated as mt15982F and mt16267R, which would produce a predicted amplicon of 286 bp. The internal primers of the semi-nested PCR were designated as mt16115F and mt16267R, which would produce a predicted amplicon of 153 bp from mouse mtDNA. PCR system and setup were the same as for the *gag* gene-nested PCR study. However, PCR conditions were slightly different: 4 min at 94 °C; (30 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C) × 40–45 cycles; 10 min at 72 °C.

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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
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識別番号・報告回数			報告日	第一報入手日 2010年10月4日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②③ポリエチレングリコール処理抗破傷風人免疫グロブリン ④⑤乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	ABC Newsletter 2010#31/2010/08/27	公表国 イギリス
販売名 （企業名）	①テタノブリンIH静注250単位　（ベネシス） ②テタノブリンIH静注1500単位　（ベネシス） ③テタノブリン-IH　（ベネシス） ④テタノブリン筋注用250単位　（ベネシス） ⑤テタノブリン　（ベネシス）			

研究報告の概要

献血者からCSF患者を永久に延期（禁止）した英国
11月1日以後、慢性疲労症候群/筋痛性脳脊髄炎（CFS/ME）に罹ったことがある人の献血を永久に延期した。この決定は、2つの米国の基礎研究による調査結果に基づくものである。連邦当局の一人が、遺伝子的にマウス白血病ウイルスに類似したウイルス（xenotropic murine leukemia virus-related virus：XMRV）とCFSとの関連を裏付けた。
英国保健省の保健保護の責任者 Clara Swinson からの手紙によると、「この変更は、CFS/ME が再発状態の時、ドナーの安全を理由に行われた。それは、原因不明の他の再発状況、または神経病学的状況と結びついたCFS/MEの診療へ至る。」とするもの。
Swinson 氏によると、ドナー選択基準の変更は、（CFS/ME 専門諮問委員会と同様に）ドナーの維持と選択において、英国 BSSAC（Blood Services Standing Advisory Committee）による勧告に従った。これまで、保健省のカスタマーサービスセンターの Mary Heaton によると、CFS罹患者の体調が優れない期間のみ、献血を延期していた。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンIH静注250単位の記載を示す。
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抗体陰性であることを確認している。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破傷風抗毒素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破傷風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ウイルス除去膜によるろ過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
マウス白血病ウイルス（murine leukemia virus：MLV）は、レトロウイルス科ガンマレトロウイルスに属し、ビリオンは球形で直径約100nmの脂質エンベロープを有する比較的大きなRNAウイルスである。万一、MLVが原料血漿に混入したとしても、HIV-1をモデルウイルスとしたウイルスバリデーション試験結果から、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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ABC NEWSLETTER

CURRENT EVENTS AND TRENDS IN BLOOD SERVICES

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2010 #31 August 27, 2010

INSIDE:

Study Confirms Link Between MLV-Related Viruses and CFS

A federal research team has found a strong association between chronic fatigue syndrome (CFS) and a diverse group of murine leukemia virus (MLV)-related viruses, according to a study published online Monday by *Proceedings of the National Academy of Sciences (PNAS)*. The findings corroborate an earlier study and could have significant implications for blood centers, because the team detected MLV-like viral sequences in 6.8 percent of 44 healthy blood donors tested.

The research was carried out by Shyh-Ching Lo, MD, PhD, of the Food and Drug Administration; Harvey Alter, MD, of the National Institutes of Health Clinical Center; and five colleagues at FDA and Harvard Medical School. The results have been eagerly anticipated, because they confirm the results of a study published in *Science* in October but conflict with a number of more recent studies (see related story, below).

In the *Science* study, the research team found that the blood of 68 of 101 patients with CFS (67 percent) contained xenotropic murine leukemia virus-related virus (XMRV), a novel gamma retrovirus. It found the same virus in the blood of eight of 218 healthy people (3.7 percent). XMRV had been previously linked to prostate cancer, but not to CFS (see *ABC Newsletter*, 1/8/10). However, a number of follow-up studies in the US and around the world – the most recent was published online in *Retrovirology* on July 1 – have not been able to confirm those results. XMRV is a type of MLV-related virus.

(continued on page 3)

Conflicting Federal Study Sparked Scrutiny, Delay

Monday's release by *Proceedings of the National Academy of Sciences (PNAS)* of a study that found a correlation between chronic fatigue syndrome (CFS) and murine leukemia virus (MLV)-related viruses ends a months-long delay and a great deal of anticipation in the CFS community.

The study, led by Harvey Alter, MD, and Shyh-Ching Lo, MD, PhD, was accepted for publication in *PNAS* in May, but its publication was delayed when a team of scientists at the Centers for Disease Control and Prevention (CDC) completed a study in which they did not find XMRV in the blood of people with CFS. That team was led by William Switzer; its results were published in *Retrovirology* on July 1 (see *ABC Newsletter*, 7/2/10).

(continued on page 4)

OUR SPACE

By ABC CEO Jim MacPherson

Scary Stuff

Earlier this month America's Blood Centers' summer meeting in Chicago provided a reality check to ABC member executives – as if they needed one – about the freight train headed their way. An executive forum was held that confirmed the huge challenges ahead and the opportunities they present.

The blood community has talked for 15 years about the pressures hospitals are under to cut costs. Yet the reality of Congress' recent healthcare reform legislation is extreme pressure – starting now – on hospitals to cut costs while they are improving patient outcomes, satisfaction, and quality measures. In exchange for millions of new "paying customers" (patients who are forced to buy insurance or who are otherwise covered under new federal programs), hospitals agreed to accept more than $150 billion in Medicare cuts over 10 years (Medicare pays for more than 55 percent of healthcare costs). Medicare only pays 88 cents for every dollar spent on covered patients, and hospitals already struggle to subsidize that loss. But should they agree that Medicare reimbursement is the new benchmark to hit?

Moreover, starting in 2012 Medicare will provide incentives for hospitals to integrate with physician groups and other providers to cut costs and improve quality. As noted in this column before, such accountable care organizations (ACOs) are poorly defined, but we do know that the targets for the most intense pressure will be big states like Florida and Texas, which have the highest costs per Medicare recipient and the lowest quality of care. Does this mean that states like Wisconsin and Minnesota, which have low cost and high quality, are to be emulated? And where do blood centers fit in?

While blood centers account for less than 1 percent of hospital costs, three factors make blood centers "low hanging fruit" for efforts to reduce costs: the new perception of blood as a commodity, blood being the number one budget item for hospital labs, "bloodless" advocates saying blood is being overused. At the same time, many blood providers are gearing up for a price war to keep or regain hospital marketshare.

In many ways today's scenario is reminiscent of the late 1990s, but many elements are new and blood centers are feeling their way on a day-to-day basis. Uncertainty is always scary – and a great teacher.

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ABC is an association of not-for-profit, independent community blood centers that helps its members provide excellence in transfusion medicine and related health services. ABC provides leadership in donor advocacy, education, national policy, quality, and safety; and in finding efficiencies for the benefit of donors, patients, and healthcare facilities by encouraging collaboration among blood organizations and by acting as a forum for sharing information and best practices.

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Study Links CFS and MLVs (continued from page 1)

Methodology. Dr. Lo, Dr. Alter, and their colleagues tested serum and whole-blood samples collected from 37 CFS patients in the mid-1990s. They also tested repeat blood samples collected two years later from four of the same patients, as well as eight repeat samples collected in 2010. As a control, they tested blood samples from 44 healthy volunteer blood donors.

The scientists used nested polymerase chain reaction (PCR) assays to determine whether MLV-like virus *gag* gene sequences were present in DNA from the samples. They also used reverse transcription (RT)-PCR assays to analyze the RNA from the patients' plasma samples. They confirmed positive results by sequencing.

Findings. The scientists found MLV-like virus *gag* gene sequences in blood samples from 32 of the 37 CFS patients (86.5 percent). They found MLVs in 3 of the 44 (6.8 percent) healthy blood donors. In addition, they found that seven of the eight patients who were tested both in the mid-1990s and in 2010 were still positive in 2010.

Dr. Lo, Dr. Alter, and their colleagues also considered whether their results could have been due to contamination, but they found no evidence that mouse DNA had contaminated the PCR assay system or the clinical samples. Furthermore, the MLV-related viruses in these samples were genetically diverse, whereas contamination would have led to the same sequence in most or all of the samples.

In their discussion section, the authors pointed out that previous studies had found very similar nucleic acid sequences in the XMRV in the blood of CFS patients. In contrast, this research team found three different types of MLV-related virus *gag* gene sequences. In a conference call with reporters, Dr. Lo said the variability found in this study is consistent with typical MLV behavior. He also said that the researchers involved in the *Science* study are currently retesting their samples and finding more variability than they had originally discovered.

In their *PNAS* article, the researchers acknowledge that "the finding of XMRV or MLV sequences in persons with CFS or other diseases does not constitute definitive proof of viral infection," and they point out that "demonstrating the integration of the viral genes into the human genome" may be necessary to show viral infection. They also point out that their study reached a different conclusion about the

(continued on page 4)

UK to Permanently Defer CFS Patients from Donating Blood

As of Nov. 1, blood donors in the UK who report that they have had chronic fatigue syndrome/myalgic encephalomyelitis (CFS/ME) will be permanently deferred from giving blood. The decision appears to be based on findings by two US-based studies, one of them federal, that corroborate a link between xenotropic murine leukemia virus-related virus (XMRV) and CFS (see stories, page 1). According to a letter to colleagues from Clara Swinson, director of Health Protection for the UK Department of Health, "This change is being made on the grounds of donor safety, as CFS/ME is a relapsing condition. It brings practice for CFS/ME into line with other relapsing conditions or neurological conditions of unknown origin." According to Ms. Swinson, the change to donor selection criteria was made following a recommendation by the UK Blood Services Standing Advisory Committee on the Care and Selection of Donors, as well as its Joint Professional Advisory Committee. Heretofore, the Department of Health had deferred only people with CFS while they felt unwell, according to Mary Heaton of the Department of Health's Customer Service Center. (Source: E-mail correspondence provided by K. Kimberly McCleary, president and CEO, The CFIDS Association of America)

Study Links CFS and MLVs (continued from page 3)

possible association between XMRV, MLVs, and CFS than a number of other studies. They posit that these varied results may be caused by geographic differences in MLV-related viruses, different sensitivities in the PCR primers used in various studies, or heterogeneity in patients with CFS.

The authors also call for further studies to determine whether their results can be generalized to other patients with CFS. They conclude by emphasizing that none of the published studies have demonstrated a causal role for MLVs in CFS, and that more research will be necessary to determine whether MLV-related viruses or XMRV "might be blood-transmitted and pathogenic in blood recipients."

In an accompanying editorial, Valerie Courgnaud, PhD, and three colleagues from the University of Montpellier in France and the University of Alberta in Canada warned that the discovery of XMRV and MLVs among blood donors could suggest "a more widespread source of infection." They also point out that there may be "a variety of xenotropic and polytropic MLVs in North America," both in people with CFS and in healthy people. They also point out that "it is likely that more than one environmental agent impacts on the development of both CFS and prostate cancer." If so, that could explain why some people have MLVs but do not develop CFS or prostate cancer.

Citations. Lo SC, *et al.* Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. PNAS. 2010 Aug. 23 (E-pub ahead of print). Courgnaud V, *et al.* Commentary: Mouse retroviruses and chronic fatigue syndrome: Does X (or P) mark the spot? PNAS. 2010 Aug. 23 (E-pub ahead of print). ♦

PNAS Publishes Its Study (continued from page 1)

FDA announced the release of the *PNAS* study in a press release on Monday, and NIH hosted a telebriefing during which Dr. Alter, Dr. Lo, and officials from FDA and CDC discussed the study with reporters. *PNAS* also published two editorials with the study results.

In an editorial in *PNAS* and in an interview with *The Scientist*, *PNAS* Editor-in-Chief Randy Schekman, PhD, said the request to hold the study had come from the authors, who wanted to review their findings in light of the study in *Retrovirology*. Although the article had already been through peer review with *PNAS*, Dr. Schekman solicited another review from "an established person in the field," who recommended that the paper not be published until the researchers "could demonstrate that the virus' genes were integrated into the human genome."

According to Dr. Schekman, Dr. Alter agreed that evidence of this process would be "the highest standard that would prove the case." However, as Dr. Alter told a *Wall Street Journal* health blogger, it would take months to collect the data that shows this integration. Dr. Schekman reported that Dr. Alter was concerned that delaying the publication for that long "would be a disservice to the CFS community." (The members of that community – particularly people with CFS – are eager for any information that sheds light on the disease, as its causes and treatments are still unknown.)

Dr. Alter told the *Wall Street Journal* that the research team instead did additional work to make sure their findings were not caused by laboratory contamination, rather than the viruses. He said that extra work strengthened their findings. Finally, the researchers concluded, and another retrovirus expert agreed, that the data were strong enough to publish the study results now. The authors did, however, add a comment to the paper, explaining the need for "proof of low-grade infection by MLV-related viruses in humans . . . by demonstrating the integration of the viral genes into the human genome."

(continued on page 5)

PNAS Publishes Its Study (continued from page 4)

In his editorial, Dr. Schekman asserted that the controversy over the association between MLVs and CFS "raises important issues regarding the release of research results to the public and the need for close collaboration with the authors and funding agencies when there is a direct link to public health."

What's Next. During the NIH's telebriefing, the CDC's Steve Monroe, PhD, said the results "raise as many questions as they answer," and he pointed out that the conflicting results from various laboratories "show there are a lot of things about the virus we don't know." For example, the officials pointed out that scientists still do not know how the people in the *PNAS* study were infected.

Importantly, none of the completed studies has been able to determine whether XMRV or related MLVs actually cause CFS or whether CFS may result in increased susceptibility to MLV infections. Another unknown is whether CFS might be transmissible through blood transfusions. Related to that is whether the viruses associated with CFS are present in the blood supply. Dr. Alter said a study of 1,000 blood donors that addresses that latter point is nearing completion.

Pending the results of that and other studies, AABB in June released an Association Bulletin recommending that its member blood collectors actively discourage potential donors who have been diagnosed with CFS from donating blood or blood components. The policy was recommended by AABB's Interorganizational Task Force on XMRV, which includes representatives from several government agencies, including the CDC, FDA, and the NIH, along with blood community representatives (see *ABC Newsletter,* 5/21/10 and 6/18/10). (Sources: www.the-scientist.com/blog/display/57628/, 8/23/10; http://blogs.wsj.com, 8/24/10; www.businessweek.com, 8/24/10; www.RGJ.com, 8/17/10)

Citations: Lo SC, *et al.* Detection of MLV-related virus gene sequences in blood of patients with chronic fatigue syndrome and healthy blood donors. PNAS. 2010 Aug. 23 (E-pub ahead of print). Schekman R. Patients, patience, and the publication process. PNAS. 2010 Aug. 23 (E-pub ahead of print).

RESEARCH IN BRIEF

A new study identifies the unique mechanism that allowed pandemic H1N1 viruses originating from avian species to adapt so easily in humans. In a report in the Aug. 5 issue of *Public Library of Science Pathogens*, an international team of scientists provided new insights into the biologic processes of the influenza pandemic of 2009-2010 and revealed a genetic marker that public health officials can use to help fight future pandemics. "We have found why the pandemic H1N1 virus replicated so well in humans," co-author Yoshihiro Kawaoka, DVM, PhD, a professor of pathobiological sciences at the University of Wisconsin-Madison's School of Veterinary Medicine, told *Science Daily* (8/6/10). According to an author's summary of the paper, "Influenza viruses that originate from avian species likely have to acquire adapting amino acid changes to replicate efficiently in mammals." In most viruses of avian origin, the presence of two amino acids – lysine and asparagine – at specific sites on the polymerase PB2 protein enable the viruses to jump from the avian host and replicate easily in human cells. Though the pandemic H1N1 viruses possess an avian-like PB2 gene, they do not encode those same "human-type" amino acids – PB2-627K and PB2-701N. The lysine amino acid is present in the avian protein at a different position. The team found that a basic amino acid at position 591 of PB2 can compensate for the lack of PB2-627K and allows efficient replication of both highly pathogenic H5N1 and pandemic H1N1 viruses in mammalian species. The team also documented the structure of the C-terminal portion of a pandemic H1N1 PB2 protein. The structural data, said Dr. Kawaoka, shows how the virus

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RESEARCH IN BRIEF (continued from page 5)

interacts with the host cell, and that could lead to antiviral agents for future flu viruses that use the same amino acid "trick" to infect humans. The structure was derived from an X-ray crystallographic study produced by the Seattle Structural Genomics Center for Infectious Disease, a consortium of Washington state-based organizations. The H1N1 virus, Dr. Kawaoka said, is actually a combination of four different avian and swine flu viruses that have emerged during the past 90 years, and it includes some genetic material from the 1918 pandemic virus, an influenza that killed as many as 20 million people. The H1N1 pandemic in 2009 and 2010 made as many as 34 million Americans ill and caused up to an estimated 6,000 deaths in the US. According to the World Health Organization, H1N1 had killed at least 18,398 people worldwide as of July 25. (Sources: *Science Daily*, 8/6/10)

Citation: Yamada S, *et al.* Biological and structural characterization of a host-adapting amino acid in influenza virus. PLoS Pathog. 2010 Aug 5;6(8): e1001034. ♦

Financial Realities a Focus at ABC's Interim Meeting

At America's Blood Centers' summer meeting this month, the message was clear: independent, community blood centers are operating in an increasingly competitive environment, and they will need to react nimbly in order to weather the changing conditions.

Blood centers are being pressured by hospitals and hospital chains – themselves facing healthcare-reform cost-cutting – to drop blood prices or lose market share to larger blood organizations. According to speakers at the Executive Leadership Forum on Aug. 9, the result of this state of affairs could be lower blood prices, fewer client hospitals, and further consolidation among blood centers. Though the situation is alarming, the speakers also suggested strategies that could help blood centers strengthen their relationships with their hospitals and deal with the challenges.

The Current Situation. During the morning session, Maureen Swan, of MedTrend Inc., focused on the financial implications of healthcare reform; Mindy Weinberg, a senior quality assurance analyst with the University of Pittsburgh Medical Center (UPMC), discussed how blood products fit into the supply chain at her hospital; and Robert Carden, PhD, president and CEO of Virginia Blood Services, focused on the economics of blood banking from blood centers' perspectives.

The key issue, which surfaced repeatedly, is the financial pressure being felt by hospitals and blood centers. As Ms. Swan pointed out, hospitals are facing pressure from two directions: government healthcare reform (which means less Medicare money for hospitals, as more people become eligible for Medicaid) and marketplace changes (which most likely will include new payment methods that place more emphasis on value and outcomes, rather than paying "per click," or per procedure).

Blood centers, too, are feeling financial pressure from multiple directions, as Dr. Carden made clear. On one hand, increased collections of red blood cells (RBCs) mean that supply is up. On the other hand, demand is down, in part because of better technologies (such as inventory management systems) and more conservative transfusion practices. The increase in the number of people with health insurance may drive demand for blood and blood products up, Ms. Swan said, but more and more people have high deductible plans, which encourage people to use less care and means that hospital volume drops. The net effect of these changes is therefore difficult to predict, she said.

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No. 23

医薬品 研究報告 調査報告書

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研究報告の概要

○関連供血者と受血者のサンプル分析により確認された、赤血球輸血によるパルボウイルスB19(B19)感染

背景:B19の極めて高いウイルス血症レベルが、急性感染した無症候性の供血者に認められた。単一供血者からの血液成分の輸血による感染報告はまれであるが、前向き研究を行わない限り、そのような症例が検知されない可能性がある。そこで輸血による感染リスクを調査するため、供血者ー受血者サンプルを用いて前向き研究を行った。

方法:受血者のB19 DNAについてはPCR法、B19 IgG抗体についてはEIA法を用いて検査を行った。輸血に関連しない感染を除外するため、B19 DNA陽性受血者の輸血前サンプル及び関連供血者サンプルのB19 DNA、IgG、IgM抗体を検査した。感染の確認のためにDNA配列解析と系統発生解析を行った。

結果:受血者869人のうち14人(1.6%)がB19 DNA陽性であったが、そのうち1人(0.12%)が輸血による感染と確定された。この受血者は、急性感染した供血者からの赤血球(1単位中5×10^{10} IU のB19 DNAを含む)及び他の供血者3名からの赤血球(1,320 IUのB19 IgG抗体を含む)を輸血されていた。

結論:0.12%と感染率は低いが、米国において毎年数百～数千例の感染症が発症する可能性がある。ほとんどの場合無症候性であるが、新生児や免疫不全状態、溶血状態にある者の場合、重篤になることがある。

報告企業の意見	今後の対応
パルボウイルスB19の、血液成分の輸血による感染のリスクを調査するため供血者ー受血者サンプルを用いて前向き研究を行ったところ、0.12%の感染率であったとの報告である。	今後も引き続き、ヒトパルボウイルスB19に関する新たな知見及び情報の収集に努める。日本赤十字社では、以前よりRHA法によるB19抗原検査を導入、ウイルス量の多い血液を排除してきた。また、2008年には検査法をより感度の高いCLEIA法に変更した。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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

35

MedDRA/J Ver.13.1J

TRANSFUSION COMPLICATION

Parvovirus B19 infection transmitted by transfusion of red blood cells confirmed by molecular analysis of linked donor and recipient samples

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BACKGROUND: Extremely high viremic levels of parvovirus B19 (B19V) can be found in acutely infected, but asymptomatic donors. However, reports of transmission by single-donor blood components are rare. In this prospective study, paired donor-recipient samples were used to investigate the transfusion risk.

STUDY DESIGN AND METHODS: Posttransfusion plasma or blood samples from recipients were tested for B19V DNA by polymerase chain reaction, generally at 4 and 8 weeks, and for anti-B19V immunoglobulin (Ig)G by enzyme immunoassay, at 12 and 24 weeks. To rule out infection unrelated to transfusion, pretransfusion samples and linked donor's samples for each B19V DNA–positive recipient were assayed for B19V DNA and anti-B19V IgG and IgM. To confirm transmission, sequencing and phylogenetic analysis were performed.

RESULTS: A total of 14 of 869 (1.6%) recipients were B19V DNA positive, but only 1 of 869 (0.12%; 95% confidence interval, 0.0029%-0.6409%) was negative for B19V DNA and anti-B19V IgG before transfusion and seroconverted posttransfusion. This newly infected patient received 5×10^{10} IU B19V DNA in one red blood cell (RBC) unit from an acutely infected anti-B19V–negative donor in addition to RBCs from three other donors that cumulatively contained 1320 IU of anti-B19V IgG. DNA sequencing and phylogenetic analysis showed that sequences from the linked donor and recipient were identical (Genotype 1), thus establishing transfusion transmission.

CONCLUSIONS: The 0.12% transmission rate documented here, although low, could nonetheless result in hundreds or thousands of infections annually in the United States based on calculated confidence limits. Although most would be asymptomatic, some could have severe clinical outcomes, especially in neonates and those with immunocompromised or hemolytic states.

Parvovirus B19 (B19V) is a small, nonenveloped, DNA virus of the *Erythrovirus* genus in the Parvoviridae family. It resists viral inactivation procedures commonly used in the manufacture of pooled plasma products and is the only parvovirus shown to be pathogenic in humans.

When B19V infects erythropoietic progenitors, transient but significant red blood cell (RBC) hypoplasia or aplasia ensues. This is especially common in children, but it also occurs in seronaive adults who have coexisting hemolytic anemias, such as sickle cell disease, hereditary spherocytosis, or RBC enzymopathies (see review[1]). Furthermore, B19V-induced RBC aplasia is seen in patients

ABBREVIATIONS: B19V = parvovirus B19; CBER = Center for Biologics Evaluation and Research; EOS = end of study; TRIPS = Transfusion-Related Infections Prospectively Studied; WB = whole blood.

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with congenital and acquired immunodeficiency because high-titer viremia in the absence of an effective humoral immune response can result in prolonged hypoplastic anemia. When B19V infection occurs during pregnancy, hydrops fetalis and fetal loss can result. Arthropathy, neutropenia, and thrombocytopenia have been reported in both children and adults.[1]

B19V transmission occurs through the respiratory route, vertically from mother to fetus, and through transfusion or transplantation—most often during the viremia that precedes clinical presentation. Household, day care, and school transmissions are common. Viral levels as high as 10^{13} genome equivalents (geq)/mL are often found in the blood of asymptomatic individuals during the early phase of acute infection.[2] There are numerous reports of transmission by pooled plasma-derived products, including clotting factor concentrates despite solvent/detergent (S/D) treatment, heat treatment, and/or other viral inactivation methods.[3-8] Infectivity has been correlated with a high concentration of B19V prompting plasma fractionators to implement screening for B19V DNA by nucleic acid testing (NAT) to exclude high-titer donations from entering manufacturing pools.[9-11]

The presence of anti-B19V IgG in the recipient or product may play a role in attenuating transmission. The prevalence of anti-B19V IgG exceeds 50% in some donor populations.[12,13] These antibodies are considered to be neutralizing[14,15] and to confer lifelong immunity.[1] The prevalence of B19 viremia in blood and plasma donors ranges from 0.003% to 0.88%[16-20] depending on the sensitivity of the NAT method employed and whether the testing is performed at the time of an epidemic. Low levels of B19V DNA, ranging from 10 to 10^3 IU or geq/mL, coexist with anti-B19V IgG and may persist for 3 to 5 years in immunocompetent blood donors.[21-24] Infectivity is presumably dependent on the relative balance between viral and neutralizing antibody titers, albeit the minimum infectious dose of B19V DNA, with or without coexisting anti-B19V IgG, is unknown. The IgG antibodies from multiple plasma donors are generally sufficient to render pooled plasma products noninfectious if no donor in the pool has high-level viremia. This provides the rationale for excluding only those plasma donations for further manufacturing that have viral titers exceeding approximately 10^6 IU/mL.[11,25] To date, universal blood donor screening for B19V NAT is not performed in the United States. Case reports of transmission by single-donor blood components are rare. Only four cases of transfusion-transmitted B19V-induced anemia have been documented.[16,26-28] The rarity of such case reports may reflect the fact that most infected individuals are asymptomatic and hence undetected unless enrolled in a prospective study. In this article, we describe a case of transfusion-transmitted B19V infection identified in a prospective study designated TRIPS (Transfusion-Related Infections Prospectively Studied). Pre- and serial posttransfusion samples from recipients and linked donor specimens were collected and placed in frozen storage. This permitted an estimate of the frequency of B19V infection by blood components and the infectious dose relative to the titer of antibody. Further, linked donor-recipient samples allowed for confirmation of causality by DNA sequencing and phylogenetic analysis.

MATERIALS AND METHODS

Patient population and study specimens

The TRIPS repository was initiated in November 2001 and is composed of linked donor-recipient specimens from transfusion recipients enrolled at the NIH Clinical Center (Bethesda, MD) and the Children's National Medical Center (Washington, DC) and from Suburban Hospital (Bethesda, MD). Informed consent was obtained from all donors and recipients in accordance with the Declaration of Helsinki for participation in NIH-sponsored and institutional review board–approved protocols (NIH Protocol 01-CC-0231; Children's National Medical Center, Protocol 2540). Human subjects were assigned a code number, and samples for testing were identified only by that code; the testing laboratories, including the parvovirus testing laboratory at the Center for Biologics Evaluation and Research (CBER), FDA, had no capability of linking the code number to the study participant's name. Thus far, pretransfusion plasma and/or whole blood (WB) samples from 869 enrolled recipients have been collected, generally at 4, 8, 12, and 24 weeks posttransfusion (and/or at the end of study [EOS] for repeatedly transfused subjects followed longer than 24 weeks). WB and/or plasma samples from donors and recipients were stored in 1- to 2-mL aliquots at –80°C in a central repository (SeraCare BioServices, Gaithersburg, MD). Medical records review was performed to obtain details of underlying diseases and clinical circumstances surrounding the transfusion.

Detection and quantitation of B19V DNA by polymerase chain reaction

B19V DNA levels were determined on the first available samples collected after transfusion from each recipient, usually at 4 and 8 weeks, but occasionally also at 2 weeks. DNA was extracted from 0.2 mL of plasma or WB, and B19V DNA was detected and semiquantified by an in-house nested polymerase chain reaction (PCR) method as described previously.[6] A final amplified product of 243 bp was obtained extending from Nucleotide 2951 to Nucleotide 3193, thus covering the junction of the VP1/VP2 region based on the nucleotide numbering of the published B19 Au sequence[29] (GenBank M13178). The first WHO International Standard for B19V DNA[30] (NIBSC

99/800, 10^6 IU/mL when reconstituted) was diluted 10^3-fold and used as a positive control for extraction and quantification. The level of B19V DNA, expressed as IU/mL, was determined by limiting dilution analysis since the conversion ratio from a geq (or a copy) to IU detected by PCR was 1:1 based on our PCR method. The sensitivity of the PCR assay with an original sample volume of 0.2 mL was 20 IU/mL, as previously described.[8] This B19V PCR procedure detects both Genotypes 1 and 2 of B19V, but not the Genotype 3 variant (see Discussion).

A posttransfusion sample was considered B19V DNA positive only when a separate 0.2-mL sample aliquot was also positive. Only if B19V DNA was positive after transfusion was the pretransfusion sample from that recipient requested from the repository, and a 0.2-mL aliquot was similarly tested by PCR. If the recipient's pretransfusion sample tested B19V DNA negative, suggesting the possibility of transfusion transmission, samples from the linked donor(s) plasma or WB were tested for B19V DNA.

Anti-B19V antibody assays

Anti-B19V IgG testing was performed qualitatively according to the manufacturer's instructions on 12- and 24-week (or EOS) samples with an FDA-cleared enzyme immunoassay (EIA) kit (Biotrin International Ltd., Dublin, Ireland) consisting of wells coated with recombinant B19 capsid protein (VP2). Index values of less than 0.9 are deemed negative and more than 1.1 are positive, while those between 0.9 and 1.1 are equivocal. The index value is obtained from the mean absorbance value for the test sample divided by the cutoff value, which is, as instructed by the kit, computed by multiplying the mean absorbance of the calibrator by the lot-specific constant. Further, we developed a semiquantitative assay to measure anti-B19V IgG levels that permitted calculating an infectious dose when B19V DNA and anti-B19V IgG were both present. This assay made use of a working standard solution consisting of 1 IU/mL anti-B19V IgG prepared by diluting the first WHO anti-B19 serum IgG standard[31] (NIBSC 93/724, 100 IU of anti-B19V IgG/mL when reconstituted) with sample diluent from the kit. A six-point standard curve was set up by twofold serial dilutions of the WHO working solution, providing a range from 0.031 to 1 IU/mL. Each plasma sample was diluted 30-fold or more, and then twofold serial dilutions were made. Aliquots of 100-µL of diluted standard or plasma solutions were incubated with the coated wells according to the manufacturer's instructions. Anti-B19V IgG levels in IU/mL were calculated by using a parallel-line model in statistical analysis software (CombiStats, Version 4.0) provided by the European Directorate for the Quality of Medicines & HealthCare (EDQM), Council of Europe (Strasbourg, France).

If recipients were confirmed B19V DNA positive (i.e., ≥20 IU/mL) from the first available sample collected after transfusion, anti-B19V IgM assays were performed on all plasma samples collected from the recipient, along with the associated donor samples. A B19V IgM EIA kit (Biotrin) was used to detect the presence of captured antibodies in human serum or plasma by means of biotinylated B19V VP2 protein according to the kit's instructions with similar computation of index values mentioned above for anti-B19V IgG testing. When a sample was scored as anti-B19V IgM equivocal, the same sample was retested for confirmation since plasma collected within 1 to 2 weeks of the initial reactive result was not available for retesting as recommended by the kit manufacturer.

DNA sequencing and phylogenetic analysis

Extracted DNA samples were amplified by a seminested B19V PCR procedure described previously[8] so that a longer final amplified product of 786 bp (Nucleotides 2408-3193), covering the entire VP1-unique region and a portion of the VP2 region, could be obtained. The amplified product was further purified by a PCR purification kit (QIAquick, Qiagen, Inc., Valencia, CA) and directly sequenced without the need for cloning since the samples sequenced contained relatively high levels of B19 DNA. The sequencing primers were the same as those used for the seminested PCR procedure. The WHO B19V DNA standard was similarly extracted and amplified, and the amplified product was sequenced for comparison. Sequences, each 710 nucleotides in length corresponding to Nucleotides 2465 to 3124 of the VP1-unique region plus Nucleotides 3125 to 3174 of the VP2 region, were aligned against other corresponding published sequences from GenBank by using ClustalW2, a general-purpose multiple alignment program. Phylogenetic analysis was performed with a neighbor-joining algorithm in computer software (Molecular Evolutionary Genetics Analysis, v.4, MEGA4, Center of Evolutionary Functional Genomics, Arizona State University, Tempe, AZ).

Statistical analysis

Statistical software (StatXact, v.8, Cytel, Inc., Cambridge, MA) was used to calculate 95% confidence interval (CI) based on one observed B19V infection in this study.

RESULTS

Fourteen of the 869 (1.6%) recipients were found to be B19V DNA positive in their early posttransfusion specimens; of these, seven were children and seven (Recipients 3-6, 8, 12, and 14 in Table 1) were adults. Pretransfusion and serial posttransfusion plasma samples from these 14 B19V DNA–positive recipients were tested for viral levels and IgM/IgG anti-B19V antibodies, and the results are shown in Table 1. Six-

TABLE 1. Analysis of 14 recipients positive for B19V DNA after transfusion*

	Before transfusion			After transfusion		
	B19V DNA	B19V antibodies		B19V DNA†	B19V antibodies‡	
Recipient	(IU/mL)	IgG	IgM	(IU/mL)	IgG	IgM
1	63	Positive	Negative	20	Positive	Negative
2	20	Positive	Negative	40	Positive	Negative
3	20	Positive	Negative	58	Positive	Negative
4	60	Positive	Negative	40	Positive	Negative
5	20	Positive	Negative	630	Positive	Negative
6	40	Positive	Negative	63	Positive	Negative
7	Negative	Positive	Negative	20	Positive	Negative
8	632	Positive	Positive	1.4×10^3	Positive	Negative
9	6.3×10^6	Positive	Positive	6.3×10^3	Positive	Positive
10	2×10^7	Positive	Positive	630	Positive	Positive
11	$>2 \times 10^{10}$	Negative	Positive	1.4×10^4	Positive	Positive
12	63	Negative	Negative	200	Positive	Negative
13	Negative	Negative	Negative	200	Negative	Negative
14	Negative	Negative	Negative	6×10^6	Positive	Positive (12 week)

* Posttransfusion samples were tested first. Only 14 recipients tested positive and hence their pretransfusion samples were obtained from the repository and tested for all B19V markers. All other recipients whose posttransfusion samples were negative for B19V DNA (i.e., <20 IU/mL) were not further investigated.

† B19V DNA levels listed were determined from the first available samples collected after transfusion, mostly at 4 weeks, except at 8 weeks for Recipients 5, 11, and 12 and at 2 weeks for Recipients 6 and 14.

‡ For each sample, the qualitative method for detecting anti-B19V (either IgG or IgM) has been described in detail under Materials and Methods. However, a designation of positive or negative in this table refers to the results obtained for testing both 12- and 24-week (or EOS) samples with the following exceptions: anti-B19V IgM was positive only in the 12-week sample for Recipient 14; anti-B19V testing was performed only on the 12-week sample for Recipients 5, 10, and 12; only on the 24-week (or EOS) sample for Recipients 1, 6, and 13; and only on the 4-week sample for Recipient 9 because of sample availability.

recipients (Recipients 1-6) had low-level viremia and anti-B19V IgG in their pretransfusion sample, indicating prior chronic infection unrelated to the index transfusion. One recipient (Recipient 7) was anti-B19V IgG positive and B19V DNA negative before transfusion and then, in the 4-week posttransfusion sample, displayed very low-level, transient viremia that coexisted with anti-B19V IgG. This case was considered to have existing B19V infection with fluctuating low-level viremia. Three recipients (Recipients 8-10) were found positive for both B19V DNA ($\leq 2 \times 10^7$ IU/mL) and anti-B19V (both IgM and IgG) before transfusion, two (Recipients 9-10) showing diminishing titers after transfusion and one (Recipient 8) showing a slight increase in titer. These three patients appear to have had an acute B19V infection that predated the transfusion. Recipient 11 clearly had an acute B19 infection that predated the transfusion since the pretransfusion sample had a very high B19V DNA level ($>2 \times 10^{10}$ IU/mL) associated with IgM antibody in the absence of IgG; after transfusion the viral level diminished and the patient seroconverted for anti-B19V IgG. Recipient 12 appeared to be in the seronegative window period of infection before transfusion since only low-level B19V DNA was detected before transfusion, and seroconversion for anti-B19V IgG was demonstrated after transfusion. Recipient 13 was negative for all B19V markers before transfusion; a single posttransfusion sample at 4 weeks had a B19V DNA level of 200 IU/mL, but later samples were negative and there was no evidence of antibody seroconversion. We considered this more likely a false-positive DNA determination than a transfusion-associated infection. Thus, only Recipient 14 fulfilled the criterion for a transfusion-related infection in that the recipient was negative for all B19V markers before transfusion and then developed high-level B19V DNA (6×10^6 IU/mL) and underwent seroconversion for IgM and IgG antibodies after transfusion. This patient is the subject of the following case report.

CASE REPORT

The single recipient infected with B19V through transfusion during the course of this study was a 35-year-old white female with a primary diagnosis of low-grade adenocarcinoma of the appendix with peritoneal carcinomatosis. She was married, had no children, and had no prior history of receiving a blood transfusion. As part of her surgical protocol, the patient received irradiated, leukoreduced RBCs from a total of six donors, consisting of two different units given every other day over a period of 5 days. A pretransfusion sample was collected on May 23, 2005, before her surgery.

As shown in Table 2, this seronegative recipient became strongly B19V DNA positive 2 weeks posttransfusion and remained positive at 4, 8, and 12 weeks, but was negative by Week 24. Anti-B19V (IgM and IgG) were absent before transfusion and then detected in both the 8- and the 12-week posttransfusion samples. IgG antibody persisted through the 24-week sample, but IgM antibody was no longer detectable at that time.

TABLE 2. B19V DNA and anti-B19V in a recipient*

Plasma sample	B19V DNA (IU/mL)	Anti-B19V IgG	Anti-B19V IgM
Before transfusion	Negative†	Negative	Negative
After transfusion			
2 weeks	6×10^6	Negative	Equivocal‡
4 weeks	20	Negative	Negative
8 weeks	630	Positive	Positive
12 weeks	140	Positive	Positive
24 weeks/EOS	Negative	Positive	Negative

* Recipient 14 in Table 1.
† Less than 20 IU/mL B19V DNA.
‡ Retested sample was also scored as "equivocal" according to the kit's instructions.

TABLE 3. B19V marker testing of donations received by the B19V-infected recipient

Donor number	Transfusion day	B19V DNA (IU/mL)	Anti-B19V IgG (IU/mL)	Anti-B19V IgM
1	0*	NT†	NT	NT
2	0	Negative	Negative	Negative
3	2	Negative	Positive (22‡)	Negative
4	2	5×10^9‡	Negative	Negative
5	4	Negative	Positive (77‡)	Negative
6	4	Negative	Positive (33‡)	Negative

* RBC units from two donors were transfused every other day starting on Day 0, the date of surgery, for a total of 6 RBC units.
† NT = not tested; sample was not available for testing.
‡ Geometric mean titer from three independent assays.

In the complex setting of surgical blood loss and transfusion replacement, measurement of hematocrit and hemoglobin could not discern whether the parvovirus infection had a suppressive effect on RBC production. Because the infection was not recognized until stored samples were retrospectively tested, reticulocyte counts were not performed. Platelet (PLT) and white blood cell counts remained normal and hospital chart review showed no record of the temporal occurrence of fever, rash, arthritis, or cardiopulmonary dysfunction.

The recipient was transfused with 6 RBC units: 2 units during exploratory surgery and 2 units on Postoperative Days 2 and 4, respectively. Of the six donors, one had no stored sample available for testing; one was negative for all B19V markers; three donors were positive for anti-B19V IgG (22, 77, and 33 IU/mL, respectively), but negative for IgM antibody and B19V DNA; and one donor (Donor 4) was acutely infected and had high-level B19V DNA (5×10^9 IU/mL) without any detectable anti-B19V (Table 3).

We calculated the total infectious dose by making two assumptions, namely, that there was 10 mL of residual plasma per RBC unit and that the unit unavailable for testing was negative for all B19V markers. With these assumptions, we estimate that the recipient received 5×10^{10} IU of B19V DNA from the acutely infected donor's RBC unit and 1320 IU of anti-B19 IgG from three donors' units (220, 770, and 330 IU).

To confirm that the B19V-contaminated RBC unit was the source of infection, direct sequencing of PCR-amplified products from the linked, implicated donor and the recipient was performed. We chose to sequence primarily the VP1-unique region, which exhibits the most variation in both DNA sequences and protein expression.[8,32] Plasma and WB from the implicated B19V DNA-positive donor (Donor 4 in Table 3) and plasma from the 2- and 8-week posttransfusion recipient samples were extracted for DNA and amplified by PCR using primers encompassing the entire VP1-unique region and a portion of the VP2 region. The amplified regions were sequenced and subjected to sequence alignment analysis. For comparison, a positive control (B19V DNA present in the WHO B19V DNA standard) was similarly PCR amplified and the purified-amplified product was sequenced. Identical 710 nucleotides covering the 650-nucleotide VP1-unique region and the 60-nucleotide VP2 region were obtained from the recipient's 2- and 8-week plasma samples and the donor's plasma and WB samples (Fig. 1A). In contrast, sequences similarly obtained from the WHO International Standard for B19V DNA had four nucleotide differences (C2531G, C2578T, A2736G, and T2786C) within the VP1-unique region while the published sequences of a well-known B19V strain, designated as Au,[29] differed by three nucleotide substitutions (the first three). These nucleotide differences would yield only two amino acid residue changes between the infecting strain in our patient and the WHO standard or the Au strain. By phylogenetic analysis, both the recipient and the donor were closely related on the same branch of the Genotype 1 phylogenetic tree and were distinct from the WHO standard and other known B19 isolates (Fig. 1B). Thus, in this prospective study, although 14 of 869 (1.6%) recipients were found to be B19V DNA positive, the observed transmission rate by transfusion was 1 in 869 (0.12%) with a 95% CI of 0.0029% to 0.6409%.

DISCUSSION

Of four previously reported cases of B19V transmission by single-donor blood components, three[16,26,27] were by RBC transfusions and one[28] was by PLTs. All these cases were identified by retrospective studies triggered by clinical symptoms or unexplained anemia observed in recipients who were immunocompromised and negative for all B19V markers before transfusion (pretransfusion sample not

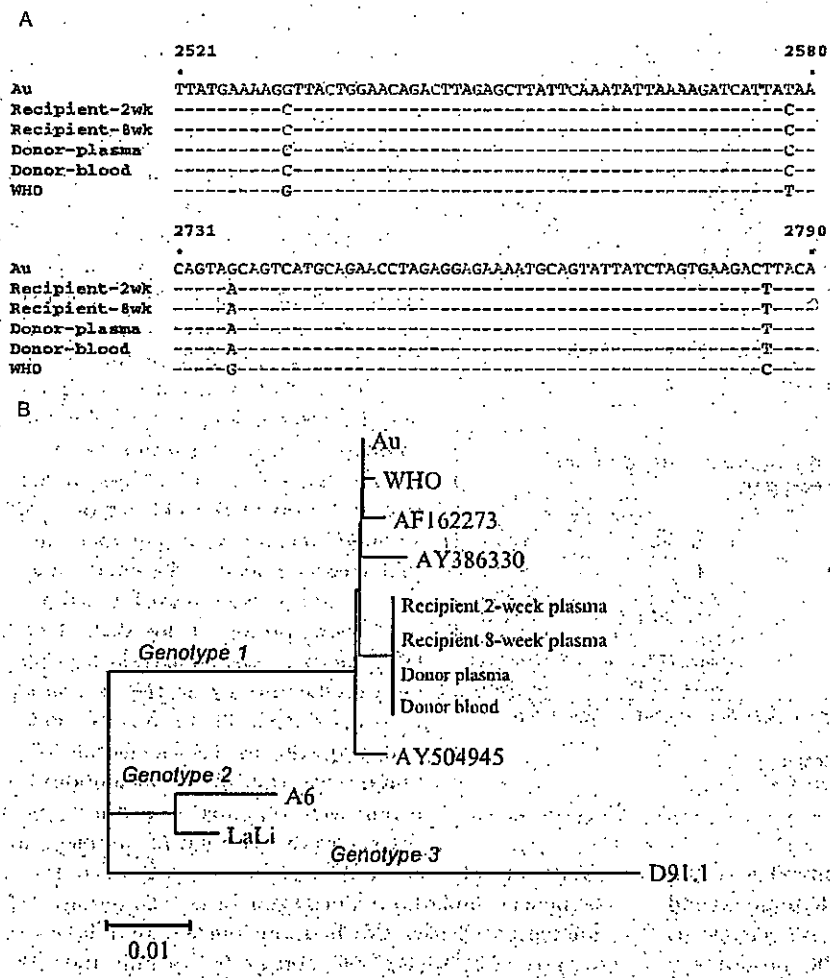

Fig. 1. (A) B19 nucleotide sequence alignments within the VP1-unique region (Nucleotides 2465-3124) and a portion of the N-terminal VP2 region (Nucleotides 3125-3174). All sequences obtained for Nucleotides 2465 to 2520, 2581 to 2730, and 2791 to 3174 were identical and therefore are not shown. Nucleotide numbering of the sequence is based on the published B19 Au strain (M13178). Sequences were determined directly from purified PCR-amplified products derived from the patient's 2- and 8-week plasma samples and from plasma and WB samples from Donor 4 (Table 3). As the positive control, the WHO B19V DNA standard was similarly sequenced. (B) Phylogenetic comparison of the above-mentioned B19 sequences (Nucleotides 2465-3174; 710 nucleotides) along with other corresponding published B19 sequences in the GenBank, that is, Genotype 1 = M13178 (Au), AF162273, AY386330, and AY504945; Genotype 2 = AY064476 (A6) and AY044266 (LaLi); and a Genotype 3 = AY083234 (D91.1). Evolutionary distances are in units of the number of base substitutions per site (0.01 unit shown).

available in one case[16]). In two studies,[25,28] the implicated donors were positive for both B19V DNA and IgM antibody, while in the third study,[16] the implicated RBC unit was B19V DNA positive in the absence of antibody. In the fourth reported case,[27] donor samples were not available. In only one study[28] was DNA sequencing and phylogenetic analysis performed to confirm that the donor was the source of infection. Furthermore, in all these case reports, detection of B19V DNA by PCR was qualitative, and hence the amount of B19V DNA infused is unknown.

Two other studies[33,34] assessed the safety of single-donor blood products containing a known amount of B19V DNA. In one,[33] 200 mL of a WB unit containing a low level (5.8×10^2 IU/mL) of B19V DNA was transfused to a seronegative (hence susceptible) pediatric recipient. The other study[34] retrospectively evaluated several adult hematologic patients who received blood components with B19V DNA levels ranging from less than 600 to 2.2×10^6 geq/mL. However, neither of the recipient populations had any clinical or laboratory evidence of B19V infection, possibly because anti-B19V IgG was present in either the transfused blood components or the recipients. Although quantitative titers of B19V IgG were not provided, the antibodies likely played a protective role in attenuating transmission.[14,15]

One recent study[35] evaluated retrospectively the rate of transmission in susceptible recipients, that is, those B19V IgG–negative individuals who received B19V DNA–positive blood components, by using linked donor and recipient repository samples established in 2000 to 2003 within the United States. A B19V DNA prevalence of 0.84% (105/12,529) was detected in those linked donations, consistent with the 0.88% prevalence[20] found earlier in unlinked donations from the same repository. Of the 105 recipients of B19V DNA–positive donations, 78% had anti-B19V IgG in their pretransfusion samples leaving only 24 susceptible recipients eligible for evaluation. No susceptible recipient was infected by transfusion; however, all received blood products containing only low-level ($<10^4$ IU/mL) B19V DNA that coexisted with anti-B19V IgG. In addition, the study design did not include early posttransfusion samples to detect transient viremia or the appearance of anti-B19V IgM. Three blood components derived from donations with higher titers of B19V DNA (>10^5 IU/mL) and devoid of anti-B19 IgG were transfused but their infectivity could not be

evaluated since each was infused into a nonsusceptible recipient.

The TRIPS study described herein is the first to investigate prospectively B19V transmission associated with transfusion of blood and blood components to susceptible immunocompromised or immunocompetent recipients. After the initial demonstration that 1.6% (14/869) of recipients had detectable B19V DNA after transfusion, the availability of pretransfusion samples allowed identification of those who were already infected with B19V. The majority had clear evidence of B19V infection existing before entry into the study and, in one case, evidence of a very recent infection that might have been attributed to transfusion if the appropriate pre- and posttransfusion samples had not been available.

Of the 14 recipients who were viremic after transfusion, only one was seronegative before transfusion and subsequently exhibited a seroconversion profile indicative of B19V transmission. This occurred after receiving a unit of RBC that had a minimum volume of plasma associated with it. By assuming that approximately 10 mL of plasma was present, we estimated that the patient received 5×10^{10} IU of B19V DNA. In reports of seronegative persons with hemophilia infused with contaminated coagulation concentrates[3] or seronegative volunteers experimentally exposed to B19V,[36] viral DNA was usually detected within 1 week after exposure. In the latter study,[36] peak viremia and seroconversion to anti-B19V IgM occurred within 2 weeks. The recipient infected in our study exhibited maximal viremia (6×10^6 IU/mL) 2 weeks after transfusion, but anti-B19V IgM was not detected until Week 8, simultaneous with the appearance of anti-B19V IgG. This delay might have been due to the modulating effect of B19V IgG–neutralizing antibodies present in three other RBC units transfused in close proximity to the implicated unit.[1,14,15]

Sequencing/phylogenetic analysis established that both the donor and the recipient were infected with B19V Genotype 1. Although B19V strains are genetically more diverse than previously thought and have been classified into three genotypes,[37] Genotype 1 is still the most prevalent in western countries while Genotype 2, though less common, has been detected in plasma and coagulation factor concentrates.[38-40] Genotype 3 is found predominantly in West Africa[41] and rarely in other areas of the world. Because some B19V NAT assays detect only Genotype 1,[40,42] some earlier investigations may have underestimated the diversity of B19V in the specimens examined. Recently a source plasma donor infected with a Genotype 3 strain was identified in the United States by a plasma fractionator performing a B19V NAT screening procedure capable of detecting all three genotypes in a minipool format.[43] The NAT procedure used in our study detects both Genotypes 1 and 2, but not Genotype 3.[11] Interestingly, B19V genotype variants have been shown to be very similar in functional and immunologic studies, and current data suggest that only one serotype exists for B19V.[38,41,44]

In our study, the infectious dose received by the susceptible (seronegative) recipient was 5×10^{10} IU of B19V DNA from 1 RBC unit (derived from an acutely infected donor) given in temporal proximity to a total of 1320 IU of anti-B19V IgG antibodies from 3 other RBC units. In the B19V transmission incident associated with pooled plasma, S/D treated, a pooled plasma product known to contain anti-B19V IgG,[9,45] the infectious dose received by susceptible immunocompetent volunteers was more than 2×10^9 geq or IU of B19V DNA, that is, 200 mL per implicated lot containing more than 10^7 geq/mL. In contrast, those same susceptible individuals were not infected when each received a dose of less than 2×10^6 IU of B19V DNA from product lots containing less than 10^4 IU/mL, indicating that infectivity is dependent on the balance between viral and neutralizing antibody titers. Moreover, when recipients were seropositive before transfusion, with anti-B19V IgG levels ranging from 19 to 39 IU/mL, the high-titer implicated product (i.e., that with $>10^7$ IU of B19V DNA/mL) was not infectious. Thus, there must be a specific level of anti-B19V IgG that confers protection against infection.[45] The lowest infectious dose reported was 2×10^4 IU of B19V DNA administered to a susceptible immunocompetent individual who received a Factor (F)VIII coagulation product devoid of any detectable anti-B19 IgG.[8]

The case found in our study represents the fifth documented B19V infection transmitted by cellular blood products and the fourth transmitted by RBCs. Although the observed incidence rate of 0.12% (1/869) is relatively low, it could translate to 4800 transfusion-transmitted B19V infections annually among the approximately 4 million blood recipients in the United States. However, because only one definite B19V transmission was observed in this population, the 95% CI for the incidence rate is very broad (0.0029%-0.6409%) and the number of projected cases could be as low as 116 or as high as 25,636. The proportion of such infections that would have serious clinical consequences is unknown, but clearly neonates, persons with congenital and acquired hemolytic anemias, and immunocompromised patients would be at increased risk of untoward clinical events.

Currently, most plasma fractionators in the United States perform minipool B19V NAT screening as an in-process control to detect and exclude donations with B19V DNA levels of approximately 10^6 IU/mL or higher so that the level of B19V DNA in manufacturing plasma pools destined for the production of plasma derivatives will not exceed the limit of 10^4 IU/mL.[11,46] In Europe, a similar limit has been in place for plasma pools used for manufacturing anti-D immunoglobulins and plasma treated for virus inactivation.[40,47-49] It is recommended that all B19V NAT

procedures detect all three B19V genotypes.[46,50] In addition to screening, viral inactivation and removal procedures have been incorporated into the manufacture of plasma derivatives. The net effect of screening and virus inactivation when combined with complexing and/or neutralizing anti-B19 IgG antibodies that are invariably present in large plasma pools is that the final products contain little or no infectious virus. This has been confirmed in a recent survey of FVIII concentrates.[11]

In contrast to pooled plasma products, WB donations are not tested for B19V DNA, and no viral inactivation procedure is in place for blood components in the United States. The fact that screening for B19V DNA is not performed is due both to the lack of licensed commercial assays and to the absence of compelling evidence for significant clinical risk. Nonetheless, it is legitimate to ask whether the small, but finite risk could be significantly reduced by testing WB donations and withholding units that exceed a threshold level of B19V DNA (e.g., that applied to units of plasma used for manufacturing pooled products) without compromising the supply of blood components. Some blood centers in Germany and Austria have screened WB units by a B19V minipool real-time NAT procedure for several years. Blood components associated with donations having 10^5 IU/mL or more, regardless of whether they contain anti-B19V IgG antibodies, are discarded to protect at-risk individuals, whereas units with less than 10^5 IU/mL are released because of the apparently universal coexistence of anti-B19V IgG.[19] In a subsequent retrospective, linked donor-recipient infectivity study,[51] preliminary data indicate that nearly 50% (7/15) of recipients transfused with RBC units from WB donations having more than 10^5 IU/mL of B19V DNA were B19V infected, and the link between the B19V donor and recipient was demonstrated by sequence analysis. In contrast, none of 16 recipients transfused with RBC units containing less than 10^5 IU/mL B19V DNA was infected. Thus, in that study, WB screening with a threshold level of 10^5 IU/mL seems to have been warranted.

In conclusion, this study revealed a new B19V infection related to single-donor blood products; only the fifth such case in the literature. In the absence of an obvious clinical syndrome, detection of B19V infection depended on the availability of pre- and posttransfusion samples to differentiate new from existing infection. The transmission was confirmed by sequencing and phylogenetic analysis of linked donor-recipient samples and demonstrated an identical Genotype 1 sequence over the 710-nucleotide skein analyzed. Despite the low rate of transmission documented in this study, this incidence of new transfusion-transmitted B19V infections could result in numerous infections annually, some of which would have clinical consequences in susceptible populations. The introduction of B19V NAT screening of WB with a threshold level of 10^5 or more or 10^6 IU/mL or more would have interdicted transfusion of the product from this donor acutely infected with B19V and avoided this proven transmission. However, the decision to introduce universal B19V donor testing is complex and needs to be guided by additional prospective studies or further retrospective analyses of repository samples from prior studies with appropriate donor-recipient linkage.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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No. 15

医薬品 研究報告 調査報告書

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一般的名称	人赤血球濃厚液	研究報告の公表状況	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)	Zou S, Foster GA, Dodd RY, Petersen LR, Stramer SL. J Infect Dis. 2010 Nov 1;202(9):1354-61.	米国

研究報告の概要

○供血者スクリーニングによりウイルス血症と特定された者におけるウエストナイル熱の特徴

供血者のNAT検査は、症状が発現する以前のウエストナイルウイルス(WNV)感染者の特定や症状の特徴付けを可能とした。2003年6月から2008年にかけて米国赤十字は、初回検査でWNV RNA陽性となった血液供血者1,436名について、経過観察と追跡調査を行ったところ、821名の供血者がWNV感染症であると確認され、残りの者は未確定または偽陽性であった。WNV感染症の症状は576名の初期WNV感染者と、未感染者の間で比較された。それによるとWNV感染者の26%に、8つの症状(新しい発疹、全身の虚脱感、頭痛、重篤な筋肉痛、関節痛、発熱、悪寒、眼痛)のうち少なくとも3つが存在すると推定された。

症状を有する患者の半数近くが治療を求めたが、医療機関の認識不足のためWNV感染の診断を受けた者はわずか5%であった。また、女性および高いウイルス量の者は他の被験者より症状が発現する可能性が高かった。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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
米国赤十字が2003年6月から2008年にかけて、ウエストナイルウイルスRNA陽性になった供血者の経過観察及び追加検査を行い、症状の特徴が明らかになったとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の国内発生に備え、平成17年10月25日付血液対策課発事務連絡に基づく緊急対応(献血制限、NAT検査)のほか、厚生労働科学研究「献血血の安全性確保と安定供給のための新興感染症等に対する検査・スクリーニング法等の開発と献血制限に関する研究」班と共同して対応について検討している。今後も引き続き情報の収集に努める。

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MAJOR ARTICLE

West Nile Fever Characteristics among Viremic Persons Identified through Blood Donor Screening

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Nucleic acid testing (NAT) of blood donors provides opportunities for identifying West Nile virus (WNV)–infected persons before symptoms develop and for characterizing subsequent illness. From June 2003 through 2008, the American Red Cross performed follow-up interviews with and additional laboratory testing for 1436 donors whose donations had initial test results that were reactive for WNV RNA; 821 of the donors were subsequently confirmed to have WNV infection, and the remainder were unconfirmed or determined to have false-positive results. Symptoms attributed to WNV infection were determined by comparing symptom frequency among 576 donors identified with early WNV infection (immunoglobulin M antibody negative) and those with unconfirmed infection. We estimate that 26% of WNV-infected persons become symptomatic, defined by the presence of at least 3 of 8 indicator symptoms. Nearly one-half of symptomatic persons sought medical care; only 5% received a diagnosis of WNV infection. Female subjects and persons with higher viral loads detected in the index donation were more likely than other subjects to develop symptoms.

The constellation of symptoms referred to as West Nile fever (WNF) is by far the most commonly recognized clinical manifestation of West Nile virus (WNV) infection [1–3]. Although studies indicate that <1% of persons infected develop neuroinvasive disease [4–6], a serological survey conducted after the 1999 New York City outbreak indicated that 21% develop febrile illness after WNV infection [6]. However, this estimate was based on the identification of only 6 (32%) of 19 seropositive persons reporting recent febrile illness, compared with 70 (11%) of 648 seronegative participants.

The subsequent implementation of WNV blood donation screening by nucleic acid test (NAT) in the United States and Canada began in June 2003 and provides a unique opportunity to identify many WNV-infected persons very soon after infection and usually before symptom onset, thus eliminating the sample size limitations of serological surveys and the biases of clinical case detection [4, 7–12]. Three follow-up studies involving NAT-positive blood donors have studied the frequency, symptoms, and risk factors for WNF [11–13]. Brown et al [12] estimated that 30% of infected persons became symptomatic, but the study lacked a control group; Orton et al [11] estimated that 41% became symptomatic (61% minus 20% among controls); Custer et al [13] found that 34% of infected persons and 20% of controls had multiple symptoms that were compatible with WNF. These figures varied in part because of different definitions of WNF and different time periods studied surrounding the index donation. One study found that lower age and higher viral load independently correlated with symptomatic infection [12]; however, another study failed to identify a relationship between age and symptomatic infection [13].

The study reported here extends the work of Orton et al [11] at the American Red Cross (ARC) to further define the frequency of WNF-related symptoms, to define demographic and virologic factors associated with

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symptom development, and to examine health care seeking behavior of WNV-infected persons. This study represents, to our knowledge, the largest collection of WNV infections among otherwise healthy adults across different demographic groups and geographic areas that has ever been studied.

METHODS

General approach. The study population was drawn from WNV RNA–reactive blood donors identified during routine blood donation screening from June 2003 through 2008, including those who were previously reported by Orton et al [11]. RNA-reactive donors were asked to return for a follow-up blood sample and interview. Based on subsequent laboratory testing, these donors were classified as WNV-confirmed (true positive) or unconfirmed (ie, donors with samples that had false-reactive test results during routine blood donation screening). Interviews using standardized questionnaires were conducted before completion of laboratory testing and prior to receipt of confirmatory results by donors, thus allowing a relatively unbiased assessment of symptoms, because donors did not know of their WNV confirmatory status at the time of interview. Symptoms were then compared among donors with confirmed WNV infection and donors without confirmed infection. Risk factors for symptom development among the donors with confirmed WNV infection, including viral load of the index donation, were analyzed.

Donor identification and laboratory testing. Since 2003, ARC blood donors have undergone WNV NAT screening using transcription-mediated amplification (TMA; Gen-Probe and Novartis) in minipools (MPs) of 16 donations [7, 11]. Reactive pools were resolved by individually testing each donation sample comprising the pool. Routine individual donation (ID) NAT was implemented in place of MP NAT in areas where reactive donations, defined as likely to confirm, exceeded an established trigger [7]. The triggers used to convert from MP to ID NAT in response to ongoing WNV activity progressively became more sensitive, such that currently 1 reactive donation having a high signal in a reactive MP is used to convert a defined geographic location to ID NAT [14–17]. All TMA-reactive index samples identified by ID or MP NAT were also tested for WNV RNA by research-based qualitative and quantitative polymerase chain reaction (PCR) assays (National Genetics Institute). Frozen plasma components from all TMA-reactive donations were retrieved, aliquots were prepared, and samples were tested for RNA (by PCR and TMA in replicates of up to 10) and for WNV-specific antibodies. WNV antibody testing was performed with a research immunoglobulin (Ig) M assay (Abbott Laboratories) in 2003 and a combination of US Food and Drug Administration–cleared tests for IgM and IgG starting in 2004 (Focus Technologies) according to methods described elsewhere [7]. Follow-up samples collected from consenting TMA-reactive donors were also tested for RNA (by TMA and PCR) and WNV antibodies.

Donors whose index donation samples were reactive on initial screening for WNV RNA by TMA were confirmed to have WNV infection (confirmed or true positive) through replicate RNA testing by TMA and PCR of the index donation or through observed seroconversion in follow-up testing [7, 11]. False-positive donors lacked RNA reactivity upon replicate testing of samples from the index donation using 2 different methods (TMA and PCR) and did not have IgM antibodies detected in the index donation or did not seroconvert when followed. The sensitivity of the confirmatory testing algorithm, based on index donation results by TMA, PCR, or IgM testing and verified by subsequent seroconversion (IgM or IgM with IgG), was 99% [18]. Follow-up samples were collected and questionnaires were completed within 120 days after the index donation.

Epidemiological investigation. TMA-reactive donors were notified of their initial test results by letter and were contacted at the same time to schedule a follow-up visit. The follow-up visit consisted of collection of a blood sample and a face-to-face interview conducted by trained donor counselors or donor center physicians with use of standardized questionnaires. The questionnaires gathered information regarding demographic characteristics and the presence of 14 symptoms consistent with WNV infection on the day of and/or 2 weeks after the index donation. The interviewers, using a standardized 25-question survey, also queried donors about medical care seeking, including visiting a doctor as a result of WNV-related symptoms, hospitalization because of those symptoms, and whether a WNV infection was specifically diagnosed.

Donors did not know their WNV confirmatory status at the time of interview. The final study population considered for analysis consisted of donors with completed questionnaires who either did not have confirmed infection or had confirmed WNV infection and tested IgM-antibody negative at the index donation. Because donors with confirmed WNV infection who lacked IgM antibody at the index donation donated during the earliest phase of infection and were most likely to have donated before symptoms would have developed [19], including only these donors in the study minimized potential bias attributed to symptomatic individuals who failed to donate. Preliminary analysis did, in fact, demonstrate lower post-donation symptom frequency among IgM-positive (15% reported ≥1 symptom), compared with IgM-negative (53% reported ≥1 symptom), donors with confirmed WNV RNA in their samples.

Data analysis was performed with SAS software (SAS Institute) [20]. Comparison of categorical variables was assessed using the χ^2 test. Viral load was compared with use of analysis of variance. Multivariate analysis was performed by logistic regression [21].

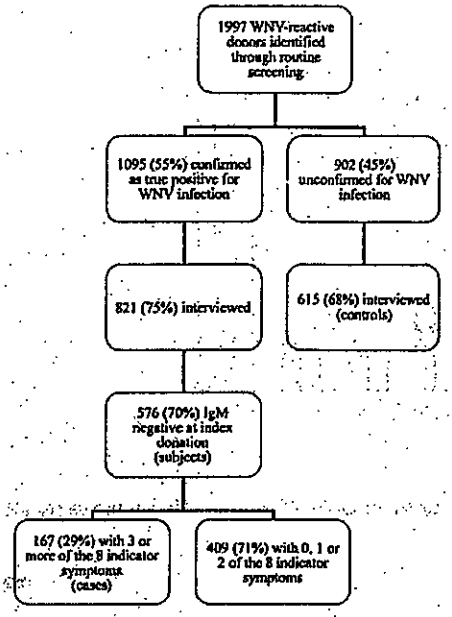

Figure 1. Study flow chart. IgM, immunoglobulin M; WNV, West Nile virus.

The ARC Institutional Review Board reviewed and approved all aspects of the study, including donor notification and consent forms for follow-up sampling and administration of the questionnaire.

RESULTS

Study population. From June 2003 through 2008, 1997 blood donors had samples that were initially reactive for WNV RNA by TMA; subsequent laboratory testing confirmed 1095 (55%) to have WNV infection, and 902 (45%) did not have infection confirmed or were found to have false-positive test results (Figure 1). A total of 966 (88%) of the 1095 donors had infection confirmed through replicate RNA testing by TMA and PCR at the time of the index donation, 116 (11%) had infection confirmed through IgM testing at the time of the index donation, and 13 (1%) had infection confirmed through IgM testing of a follow-up donation up to 63 days after the index donation (Table 1). The 1095 donors with confirmed WNV infection represented approximately one-half of the >2000 WNV-infected donors reported by all blood centers in the United States during the study period.

Completed surveys were received from 1436 (72%) of the 1997 donors. Donors confirmed as WNV RNA positive were more likely to have completed the survey (821 donors; 75%) than were donors who were not confirmed as being WNV RNA positive (615 donors; 68%; odds ratio [OR], 1.4; 95% confidence interval [CI], 1.2–1.7) (Figure 1). Seventy-eight percent of the interviews occurred within 90 days after the date of the index blood donation. There was no systematic difference in the distribution of intervals from index donation to interview between donors who were confirmed as being WNV RNA positive and those who were not confirmed as being positive (Figure 2). The frequencies of reported symptoms did not vary by duration of the follow-up interval. The final study population consisted of 576 donors who were confirmed to be positive for WNV infection and who were IgM-negative at the index donation (subjects) and 615 donors without confirmed infection (controls) (Figure 1). The remaining 245 of 821 donors with confirmed WNV infection had index donation samples that were positive for IgM. Subjects were more likely to be male (332 [58%] of 576) than were controls (292 [47%] of 615; $P < .01$).

Symptom frequency and association with WNV infection. The frequency of each symptom attributable to WNV infection was calculated by subtracting the frequency with which each symptom was reported among the 615 control donors from the frequency with which the symptom was reported among the 576 subject donors. Eight reported symptoms (new rash, generalized weakness, headache, severe muscle pain, joint pain, fever, chills and painful eyes) had an attributable frequency of >10% and had an odds of being reported by subjects that was at least 5 times the odds of being reported by controls (Table 2). Of the 576 subject donors, 270 (47%) reported none of the 8 indicator symptoms, with 85 (15%), 54 (9%), 75 (13%), 39 (7%), 23 (4%), 18 (3%), 7 (1%), and 5 (1%) reporting 1 to 8 symptoms, respectively. The symptoms were not independent from each other, judged by χ^2 analyses of each 2-symptom pair or through multivariate logistic regression analyses among subject donors, although some symptoms were more closely associated with each other (such as fever and chills or generalized weakness and severe muscle pain) than were others.

Symptomatic donors (cases) were defined as having ≥3 of

Table 1. Breakdown of Confirmatory Criteria for 1095 West Nile Virus RNA–Infected Donors from the American Red Cross, June 2003 through 2008

	No. (%) of subjects, by replicate RNA test result (TMA and PCR) at index donation	
IgM at index donation	Positive	Negative
Positive	241 (22.0)	116 (10.6)
Negative	725 (66.2)	13 (1.2)[a]

NOTE. IgM, immunoglobulin M; PCR, polymerase chain reaction; TMA, transcription-mediated amplification.

[a] Confirmed by IgM testing at follow-up 7–63 days after index donation.

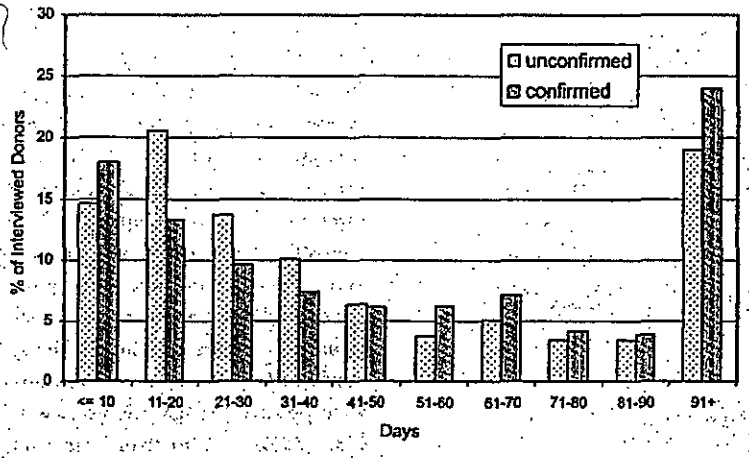

Figure 2. Distribution of intervals between index donation and interview for 821 donors confirmed as West Nile Virus RNA positive versus 615 donors without confirmed infection, American Red Cross, June 2003 through 2008.

the 8 symptoms; 167 (29%) of the 576 subjects and 20 (3%) of the 615 controls met this definition, of which the most commonly observed triad among subjects was headache, generalized weakness, and fever (55; 9.5%). Among the 167 cases, only 94 (56%) reported fever (Table 3). We estimate that 26% (29% minus 3%) of persons infected with WNV develop symptoms that meet the case definition of ≥3 of the 8 indicator symptoms due to the infection. However, it is noteworthy that 53% (306) of the subjects developed ≥1 of the 8 symptoms, compared with 11% (69) of the controls (OR, 9.0), which suggests that up to 42% of infections may result in symptoms.

Demographic characteristics and viral load. Among the 576 subjects, females (85 [35%] of 244) were more likely than were males (82 [25%] of 332) to have met the case definition for symptomatic infection ($P < .01$; OR, 1.6; 95% CI, 1.1–2.3). There was no consistent relationship between donor age and

Table 2. Frequency of Symptoms on the Day of Donation or during the 14 Days after Donation among 576 West Nile Virus–Infected Subjects and 615 Controls Identified by the American Red Cross, June 2003 through 2008

Symptom	No. (%) of donors with symptoms		Odds ratio (95% CI)	Adjusted percentage of donors reporting symptoms, % (95% CI)
	Subjects[a] (n = 576)	Controls (n = 615)		
New rash	155 (27)	6 (1)	37.4 (16.4–85.3)	26 (22–30)
Severe muscle pain	95 (16)	11 (2)	10.8 (5.7–20.5)	15 (12–18)
Painful eyes	75 (13)	9 (1)	10.1 (5.0–20.3)	12 (9–14)
Joint pain	94 (16)	11 (2)	10.7 (5.7–20.2)	15 (11–18)
Generalized weakness	159 (28)	22 (4)	10.3 (6.5–16.3)	24 (20–28)
Chills	86 (15)	13 (2)	8.1 (4.5–14.7)	13 (10–16)
Bone pain	34 (6)	5 (1)	7.7 (3.0–19.7)	5 (3–7)
Fever	109 (19)	22 (4)	6.3 (3.9–10.1)	15 (12–19)
Swollen glands	50 (9)	11 (2)	5.2 (2.7–10.1)	7 (4–9)
Headache	178 (31)	45 (7)	5.7 (4.0–8.0)	24 (19–28)
Tremor	5 (1)	1 (0)	5.4 (0.6–46.2)	1 (0–2)
New difficulty thinking	32 (6)	8 (1)	4.5 (2.0–9.8)	4 (2–6)
Vomiting or diarrhea	62 (11)	17 (3)	4.2 (2.4–7.3)	8 (5–11)
Abdominal pain	42 (7)	13 (2)	3.6 (1.9–6.9)	5 (3–8)

NOTE. CI, confidence interval.

[a] West Nile virus RNA–positive, immunoglobulin M–negative donors.

Table 3. Frequency of Symptoms on the Day of Donation or during the 14 Days after Donation among 167 West Nile Virus–Infected Subjects Meeting the Clinical Case Definition (≥3 Indicator Symptoms) Identified by the American Red Cross, June 2003 through 2008

Symptom	No. (%) of donors with symptom
Headache[a]	125 (75)
Generalized weakness[a]	125 (75)
New rash[a]	97 (58)
Fever[a]	94 (56)
Severe muscle pain[a]	90 (54)
Joint pain[a]	81 (49)
Chills[a]	79 (47)
Painful eyes[a]	67 (40)
Vomiting or diarrhea	45 (27)
Swollen glands	36 (22)
Abdominal pain	31 (19)
New difficulty thinking	29 (17)
Bone pain	27 (16)
Tremor	4 (2)

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

[a] Indicator symptom.

the development of symptoms ($P > .05$ among both males and females), and the females appeared to be more likely than males to develop symptoms regardless of age (Table 4).

Subjects meeting the case definition had higher viral loads detected in the index donation sample, compared with those not meeting the case definition ($P < .01$; Table 5). Viral loads were similar among males and females but differed among age groups ($P = .02$); donors 20–29 years of age had the lowest viral load (median viral load, 795 copies/mL; maximum viral load, 220,000 copies/mL). There was no linear trend of viral load with age for all other age groups (median viral load, 2035–7750 copies/mL with a maximum of 650,000 copies/mL). Logistic regression analysis of the relationship between viral load detected in the index donation, sex, and age and meeting the case definition showed that the odds of meeting the case definition were again significantly higher in women (OR, 1.7; 95% CI, 1.2–2.4) and increased ~1.5 times for every 10-fold increase in viral load (OR, 1.5; 95% CI, 1.2–1.8); age was not statistically significant ($P = .43$).

Medical care seeking among donors with confirmed WNV infection. Of the 167 subject donors who met the case definition during the 2 weeks after blood donation, 73 (44%) reported having visited a doctor because of their WNV-related symptoms, and 5 (3%) were hospitalized. Among the 615 controls, 38 (6%) reported having visited a doctor because of their symptoms, and 6 (1%) were hospitalized; none received a diagnosis of WNV infection. These results suggest that 38% (44% minus 6%) of donors who met the case definition had a doctor visit attributable to WNV infection, and similarly, 2% (3% minus 1%) were hospitalized as a result of their symptoms. Among the 73 patients who reported seeking medical care, only 4 (5%) of the 5 hospitalized patients received a diagnosis of WNV infection, and none received a diagnosis of neuroinvasive disease. Because interviews were conducted before final confirmatory notification (that is, donors did not know their final WNV test results at the time of the interview), all donors who sought medical care should not have been influenced by a definite diagnosis of WNV infection. Therefore, comparing subject donors who met the case definition with control donors, as was done in this study, provides an estimate of the proportion of WNV-infected individuals who seek medical care.

DISCUSSION

This study suggests that 26 percent of persons infected with WNV develop symptoms that meet our case definition and are attributable to the infection. Our case definition, which is based

Table 4. Analysis of Relationship of Donor Age to Case Definition Stratified by Sex among 576 West Nile Virus–Infected Subjects Identified by the American Red Cross, June 2003 through 2008

	Female sex		Male sex		
Age, years	No. of subjects	No. (%) of cases	No. of subjects	No. (%) of cases	OR (95% CI)
<20	15	5 (33)	14	4 (29)	1.3 (0.3–6.1)
20–29	18	7 (39)	21	4 (19)	2.7 (0.6–11.5)
30–39	26	13 (50)	33	8 (24)	3.1 (1.0–9.5)
40–49	83	30 (36)	96	30 (31)	1.2 (0.7–2.3)
50–59	63	15 (24)	98	28 (29)	0.8 (0.4–1.6)
≥60	39	15 (38)	70	8 (11)	4.8 (1.8–12.9)

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

Table 5. Viral Load among 576 West Nile Virus–Infected Subjects Identified by the American Red Cross, June 2003 through 2008

Variable	No. of subjects[a]	Viral load, geometric mean log_{10} copies/mL (± SD)	Viral load			P
			Median copies/mL	Minimum copies/mL	Maximum copies/mL	
Met the case definition[b]						
No	400	3.39 ± 1.11	1900	5	650,000	<.01
Yes	163	3.82 ± 0.99	7900	50	580,000	
Sex						
Female	241	3.53 ± 1.07	3500	5	650,000	.87
Male	322	3.51 ± 1.11	3400	5	530,000	
Age						
<20	29	3.29 ± 1.04	2035	100	230,000	.02
20–29	37	3.19 ± 1.07	795	5	220,000	
30–39	57	3.88 ± 0.79	7750	50	580,000	
40–49	175	3.61 ± 1.15	4750	5	650,000	
50–59	158	3.42 ± 1.09	2550	5	470,000	
≥60	107	3.49 ± 1.10	2850	5	530,000	

NOTE. Subjects were West Nile virus RNA–positive, immunoglobulin M–negative donors.

[a] Viral load data were only available for 563 donors.

[b] The case definition was ≥3 of the following 8 symptoms: new rash, severe muscle pain, painful eyes, joint pain, generalized weakness, headache, fever, and chills.

on the presence of at least 3 of 8 indicator symptoms (new rash, severe muscle pain, painful eyes, joint pain, generalized weakness, headache, fever, and chills), was highly correlated with WNV infection, having been met by 29% of the subjects and only 3% of the controls. Nevertheless, the estimate that 26% of WNV-infected persons become symptomatic may be low, because the presence of even 1 of the 8 indicator symptoms was highly predictive of WNV infection: ≥1 symptom was present in 53% of subjects versus 11% of controls (attributable percentage, 42%).

Our results can be compared with results from several other blood donor follow-up studies. Brown et al [12], who used a case definition of the presence of both fever and headache, estimated that 30% of infected persons become symptomatic; however, no WNV-negative control group was present for adjustment. In a study by Custer et al [13] that used a definition of symptomatic infection of ≥3 symptoms, 34% of WNV-infected donors and 20% of false-positive donors were symptomatic, which suggests that only 14% had symptoms that were attributable to WNV. It is unknown why the Custer et al [13] study had a relatively small difference observed between the percentage of subjects and the percentage of controls, which yielded a substantially lower rate of adjusted symptom reporting. This may have been related to a less rigorous laboratory-based definition of subjects and controls [13]. However, even with only 14% of individuals having symptoms attributable to WNV infection, Custer et al [13] reported a remarkably similar distribution of individual symptoms, with headache being the most common.

One notable finding of our study was the absence of reported fever among a substantial proportion of the symptomatic persons (44%). This finding was corroborated by a study involving 534 persons with WNF identified by surveillance in California, in which 31% of subjects did not report fever [22]. These findings suggest that many symptomatic WNV infections are without recognized fever.

Our data indicate that many WNV-related illnesses are clinically significant but are nonspecific and remain undiagnosed. Of the 26% of persons infected with WNV who developed symptoms meeting our case definition that were attributable to infection, we observed that 38% had visited a doctor for their symptoms, and 2% were hospitalized as a result of the infection. However, only 5% of those who sought medical care received a diagnosis of WNV infection. These results are higher than those reported in the study by Custer et al [13], which found that an adjusted 4% of individuals sought medical care (ie, 12% of all confirmed-positive donors, regardless of the number of symptoms, compared with 8% of false-positive donors). Similarly, only 2 (6%) of the 35 confirmed-positive donors who sought medical care received a diagnosis of WNV infection [13]. Only 12 (9%) of 135 viremic donors, or 29% of those meeting the study case definition for WNF, sought medical care in Colorado [12]. This low rate of recognition of WNF is consistent with surveillance data that shows that the

15,800 WNF cases reported through 2007 in the United States were a small fraction of the >300,000 infections that are estimated to have occurred [23].

Similar to the findings of Brown et al [12], we found that a higher plasma viral load at the time of index donation predicted the development of symptoms. However, there were inconsistencies with other studies regarding other possible factors associated with reported symptoms. Although Brown demonstrated decreasing proportions of infected persons developing symptoms as donor age increased, particularly among men [12], no such relationship was observed in our study or in the study of Custer et al [13]. In our study, women were more likely to have reported symptoms (OR, 1.6), similar to the report by Custer et al [13] (OR, 1.4). Nevertheless, Brown et al [12] found no relationship between sex and symptom development. We observed similar plasma viremia levels between men and women, which suggests that plasma viremia level is not responsible for increased reporting of symptoms among women.

Several limitations to our study exist. Approximately one-quarter to one-third of NAT-reactive donors did not consent to interview. If donors who developed symptoms were more likely to participate, this would bias the study towards an increased frequency of symptoms. The initial notification of preliminary WNV screening test results could have increased nonspecific symptom reporting, although there should not be a differential effect between subjects and controls. Blood donors are healthier than the general population and thus may not be representative of the entire population. However, underlying illnesses or conditions that may influence the development of WNF have not been identified in the general population. In addition, plasma viremia levels were measured only on the day of index donation and thus may not reflect peak levels or the extent of viral replication and dissemination throughout the acute infection period. Nevertheless, the time of donation in relationship to each donor's plasma viremia curve should be randomly distributed among persons within each age and sex subgroup, and thus, comparisons among these groups should reflect true population differences in viremia levels.

In summary, our data provide several insights into the clinical characteristics of WNV infection. Our data demonstrate that demographic risk factors for neuroinvasive disease strikingly contrast with those for WNF. Although surveillance data indicate that men are at greater risk than are women for neuroinvasive disease [24], our data suggest that women may be at higher risk for developing WNF. The substantial increase in the risk of neuroinvasive disease with advancing age [24] was not observed for WNF in our study. Although 26% of our WNF-confirmed study population developed symptoms that met our case definition and were attributable to infection, our analysis suggested that as many as 42% had ≥1 indicator symptom that was attributed to the infection. The fact that nearly one-half of the patients who met our symptomatic case definition sought medical care yet few received a diagnosis of WNV infection suggests that WNF has significant but largely unrecognized clinical impact [25].

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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

○ボリビアとペルーにおけるヒトへのGuaroaウイルス感染
コロンビアでは1959年に初めてGuaroaウイルス(GROV)がヒトから分離された。その後、ブラジル、コロンビア、パナマの発熱患者および蚊からウイルス分離株が採取されたが、ヒトの疾患とウイルスの関連性は不明であった。ボリビアとペルーの発熱疾患患者からGROV14株が分離され、また3症例でIgMセロコンバージョンが確認された。ペルーの都市、イキトス居住者の抗GROV抗体陽性率は13%であり、林業、漁業、油田労働等の職に就く者がもっとも陽性率が高かった。代表的なGROV分離株の遺伝子学的特性からは、ボリビアとペルーの株が、以前にブラジルとコロンビアで分離された株とは異なる可能性のある単一系統グループを形成していることが示された。本試験で、GROVが中南米の熱帯地域における発熱疾患の原因であることが確認された。

報告企業の意見	今後の対応
今までヒトの疾患とGuaroaウイルス(GROV)の関連は不明であったが、調査の結果、GROVが中南米の熱帯地域における発熱疾患の原因であることが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受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を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

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

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Guaroa Virus Infection among Humans in Bolivia and Peru

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Abstract. Guaroa virus (GROV) was first isolated from humans in Colombia in 1959. Subsequent isolates of the virus have been recovered from febrile patients and mosquitoes in Brazil, Colombia, and Panama; however, association of the virus with human disease has been unclear. As part of a study on the etiology of febrile illnesses in Peru and Bolivia, 14 GROV strains were isolated from patients with febrile illnesses, and 3 additional cases were confirmed by IgM seroconversion. The prevalence rate of GROV antibodies among Iquitos residents was 13%; the highest rates were among persons with occupations such as woodcutters, fisherman, and oil-field workers. Genetic characterization of representative GROV isolates indicated that strains from Peru and Bolivia form a monophyletic group that can be distinguished from strains isolated earlier in Brazil and Colombia. This study confirms GROV as a cause of febrile illness in tropical regions of Central and South America.

INTRODUCTION

Guaroa virus (GROV) was first isolated in Guaroa, Meta Department, Colombia, in 1959 from people without overt illness.[1] Subsequently, isolates of GROV have been made from febrile persons in Brazil and mosquitoes in Colombia, Panama, and Brazil.[2–4] Epidemiological investigations conducted in Colombia from 1956–1961 revealed that a significant number of people living in the Middle Magdalena Valley (especially adults) had GROV antibodies.[5] Ecological investigations conducted thereafter have repeatedly isolated GROV from *Anopheles (Kerteszia) neivai*; consequently, this species has been implicated as the putative mosquito vector of GROV, which constitutes a rare event for arboviruses.[4,6] Other arboviruses potentially transmitted by *Anopheles* mosquitoes include Breu Branco, Kadipiro, and Getah.[7,8]

Follow-up serologic studies in the town of Guaroa (Colombia) in 1956 indicated that 49 of 69 (75%) residents of the community had neutralizing antibodies to GROV.[1] Another serosurvey in northern Brazil (Para state) found that 18% of residents had hemagglutination-inhibition (HI) antibodies to GROV.[3] Low titers of HI antibodies to GROV have also been reported in sera of residents of Argentina, São Paulo state in Brazil, Peru, and Guatemala.[9] Collectively, the results of these studies suggest that GROV is widely distributed in Central and South America; however, its association with a specific human illness or disease syndrome remains unclear.

The virus is a member of the family *Bunyaviridae*, genus *Orthobunyavirus*; it contains a segmented negative-strand RNA genome of three segments (S, M, and L). The L segment encodes the L protein (RNA polymerase); the M segment encodes the polyprotein precursor of the virion glycoproteins, G1 and G2, and the nonstructural protein NSm, and the S segment encodes for the N and NSs proteins.[10–12] The taxonomic status of GROV has been controversial. Initially, GROV was considered to be a member of the California antigenic group (family *Bunyaviridae*) based on the results of hemagglutination-inhibition HI tests.[13,14] Subsequently, Whitman and Shope[14] showed that GROV was antigenically related to viruses in both the California and Bunyamwera serogroups. Based on the results of complement-fixation (CF) tests, GROV could be placed in the Bunyamwera group, but based on neutralization tests, it was more closely related to the California group. Because the two tests measure different gene products (nucleocapsid and glycoproteins, respectively), Bishop[15] suggested that GROV represented a reassortant virus that possesses RNA segments originally derived from Bunyamwera and California virus groups. Subsequent sequence data for the S RNA of GROV indicated that it should be classified in the Bunyamwera serogroup rather than the California serogroup.[16] In the Eighth Report of the International Committee on Taxonomy of Viruses,[10] GROV is currently classified as a unique species within the genus *Orthobunyavirus*, and it is considered distinct from viruses included in the Bunyamwera and California species complexes.

In 2000, the United States Naval Medical Research Center Detachment (NMRCD) in Lima, in collaboration with the Ministries of Health of Bolivia and Peru, initiated a passive surveillance study to investigate etiology of febrile illnesses. As part of the surveillance program in Peru and Bolivia, 17 confirmed cases of GROV infection were diagnosed in patients with acute, self-limited febrile illnesses. Nine of seventeen confirmed cases were reported in 2007 in Peru (between January and November), and two cases were also confirmed in 2007 and 2009 in febrile patients from Bolivia, providing the first evidence that GROV also circulates in that country.

In the present study, we investigated the epidemiology of GROV infection, the prevalence of GROV antibodies and risk factors for infection among residents of the Amazonian city of Iquitos, Peru, and the phylogenetic relationship among the GROV strains isolated in Peru, Bolivia, and other South American countries.

MATERIALS AND METHODS

Study sites. The confirmed GROV human cases reported in this study lived in several distinct areas of Peru, including the cities of Iquitos in the Department of Loreto, Puerto Maldonado in the Department of Madre de Dios, La Merced in the Department of Junin, and Tumbes on the coast. Iquitos

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is a city of about 380,000 inhabitants located 120 m above sea level in the Amazon Basin in northeastern Peru. Puerto Maldonado is a city of approximately 56,000 habitants located in southeastern Peru about 256 m above sea level on the banks of the Madre de Dios river near the border with Brazil and Bolivia. La Merced is a city of about 50,000 persons located 751 m above sea level in the Department of Junin. Tumbes is a Pacific coastal city located in the north near the border with Ecuador; according to the 2007 census, it had a population of 139,811 inhabitants. Two GROV isolates were also obtained from febrile patients living in Cochabamba Department in Bolivia. Figure 1 shows the approximate geographic locations of the study sites where GROV infections were confirmed.

Passive febrile surveillance study population. The study protocols were approved by the Ministries of Health of Peru and Bolivia and the Naval Medical Research Center Institutional Review Board (protocols NMRCD.2000.0006, NMRCD.2000.0008, and NMRCD.2008.0002). The study subjects were patients (> 5 years of age) who presented with a diagnosis of an acute, febrile undifferentiated illness in their home or at military or civilian outpatient clinics at the study sites. Demographic and clinical information was obtained from each patient at the time of voluntary enrollment, and a signed consent form was obtained from each subject. The criteria for inclusion in the program were fever ≥ 38°C of no more than 5 days in duration, headache, myalgia, and other nonspecific symptoms. Two paired blood samples were collected, one during the acute phase of illness and the second sample 2–4 weeks after onset of symptoms. Acute samples were tested for virus by cell culture, and both acute and convalescent samples were assayed for IgM antibodies to a variety of arboviruses (including GROV) by an enzyme-linked immunosorbent assay (ELISA), as described previously.[17] Diagnosis of a confirmed GROV infection (case) was based on isolation of the virus and/or a 4-fold or greater increase in IgM antibody titer between the acute and convalescent serum samples. A case was considered as presumptive when IgM antibodies were detected in a single acute sample or in both acute and convalescent samples without a 4-fold increase in titer.

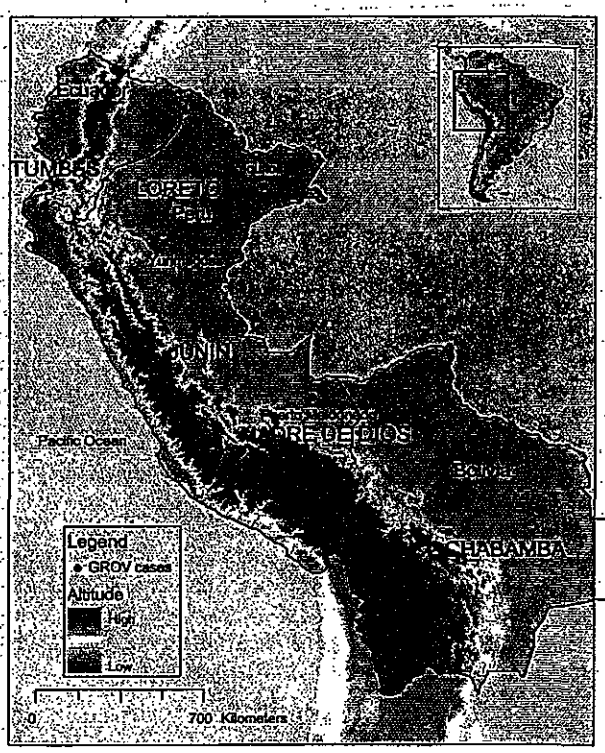

FIGURE 1. Geographic distribution of the Guaroa virus human cases identified as part of the febrile disease surveillance program. This figure appears in color at www.ajtmh.org.

Antibody prevalence studies. The antibody prevalence of GROV was determined in Iquitos by testing a total of 1,124 human serum samples for IgG antibodies to GROV by an ELISA, as previously described.[17] The samples were collected in 2006 as part of a cross-sectional antibody prevalence study carried out in Iquitos after an outbreak of febrile illness associated with Venezuelan equine encephalitis virus (VEEV) infection. Samples were collected in three Iquitos neighborhoods where Venezuelan equine encephalitis (VEE) cases were reported as well as in a control neighborhood where VEE cases were not reported.[18] Thus, the selected population represented a suitable population to test the prevalence of GROV and other arboviral diseases. Serum samples from a subset of the original study participants, who agreed to the future use of their samples, were tested. All ELISA IgG antibody-positive samples were further evaluated using an 80% plaque-reduction neutralization assay (PRNT) for GROV. Briefly, sera were heat-inactivated at 56°C for 30 minutes, and two 2-fold serum dilutions were prepared, mixed with 100 plaque forming units (PFUs) of GROV (prototype Peruvian strain OBS 0069), and incubated at 4°C overnight. The virus–serum dilutions mixtures were inoculated onto confluent monolayer of Vero cells propagated in microplates and incubated at 37°C for 1 hour before adding an overlay of 0.4% of agarose in Eagle's minimum essential medium (EMEM). After 72 hours of incubation at 37°C, the plates were stained with 0.25% crystal violet in 20% methanol, and plaques were counted. All IgG-positive samples were tested at an initial concentration of 1:20, and all positive sera were further titrated to the endpoint. Neutralization titers were considered as the highest serum dilution that reduced plaque formation by ≥ 80%.

Virus isolation. Patient's serum specimens were diluted 1:5 in EMEM, supplemented with 2% fetal bovine serum, 200 μg streptomycin, and 200 U/mL penicillin. Two hundred microliters of diluted samples were then inoculated into flasks with confluent monolayers of African green monkey kidney cells (Vero) and *Aedes albopictus* mosquito (C6/36) cells. Vero cell cultures were examined daily for evidence of viral cytopathic effect (CPE). Spot slides of C6/36 and Vero cells were subsequently prepared, and an immunofluorescence assay (IFA) was done using polyclonal antibodies against arboviruses endemic in Peru.[17,19–23] A variety of arboviruses were isolated from these samples and will be reported elsewhere. The 14 GROV isolates are listed in Table 1.

Extraction of RNA, reverse transcription, and PCR amplification of S, M, and L segments. Viral RNA was extracted using the QIAamp viral RNA mini kit (Qiagen, Valencia, CA) or Trizol reagent (Invitrogen, Carlsbad, CA) following the manufacturer's protocol. The reverse transcription reaction (RT) was done using 1× RT buffer, 0.2 mM deoxyribonucleotide triphosphate (dNTPs), 1 μM primers, 80 units RNAsin ribonuclease inhibitor (Promega, Madison, WI), 1 mM dithiothreitol, 200 U SuperScript reverse transcriptase (Invitrogen), and 5 μL RNA. The reactions were incubated at 42°C for 1 hour. The PCR

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TABLE 1
Guaroa confirmed cases included in the study

Strain	Study	Location	Year	Host	Age	Sex	Occupation	Laboratory diagnostic
CoH 352111	NA	Colombia	1956	Human	NA	NA	NA	Virus isolation*
CoAr 2526	NA	Colombia	1964	Mosquito	NA	NA	NA	*
BeH 22063	NA	Para, Brazil	1960	Human	NA	NA	NA	Virus isolation*
31498	NA	NA	NA	NA	NA	NA	NA	*
FVB 0546	Febrile surveillance	Cochabamba, Bolivia	2007	Human	17	Female	Student	Virus isolation
FVB 2032	Febrile surveillance	Cochabamba, Bolivia	2009	Human	24	Male	Agricultural worker	Virus isolation
OBS 0069	Outbreak investigations	Iquitos, Loreto, Peru	1995	Human	38	Male	Agricultural worker	Virus isolation
IQU 1091	Febrile surveillance	Iquitos, Loreto, Peru	1999	Human	30	Male	Air Force	Virus isolation
IQD 8537	Febrile surveillance	Iquitos, Loreto, Peru	2004	Human	31	Male	Agricultural worker	Virus isolation
FSJ 1266	Febrile surveillance	La Merced, Junin, Peru	2006	Human	32	Male	Agricultural worker	Seroconversion
FSJ 1318	Febrile surveillance	La Merced, Junin, Peru	2007	Human	30	Male	Driver	Virus isolation
FSJ 1335	Febrile surveillance	La Merced, Junin, Peru	2007	Human	35	Male	Agricultural worker	Virus isolation
FSJ 1340	Febrile surveillance	La Merced, Junin, Peru	2007	Human	43	Male	Driver	Virus isolation
FST 1122	Febrile surveillance	Zarumilla, Tumbes, Peru	2007	Human	27	Female	Seller	Seroconversion
FMD 1553	Febrile surveillance	Iberia, Madre de Dios, Peru	2007	Human	17	Female	House wife	Virus isolation
OBT 5637	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	21	Female	House wife	Virus isolation
OBT 5655	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	30	Male	Local healer	Virus isolation
OBT 5667	Outbreak investigations	Puerto Maldonado, Madre de Dios, Peru	2007	Human	27	Male	Miner	Virus isolation
FMD 1720	Febrile surveillance	Madre de Dios, Peru	2007	Human	17	Female	Miner	Seroconversion
FMD 1806	Febrile surveillance	Tambopata, Madre de Dios, Peru	2008	Human	37	Male	Health worker	Virus isolation
MIS 0239	Other	Puerto Maldonado, Madre de Dios, Peru	2008	Human	NA	Male	NA	Virus isolation

NA = not applicable.
* Samples provided by the World Health Organization Reference Collection, University of Texas Medical Branch (UTMB).

included 1× PCR buffer, 0.25 mM dNTPs, 1 μM primers, 3 mM $MgCl_2$, 2.5 U GoTaq DNA polymerase (Promega), and 5 μL cDNA. The conditions for the PCRs included incubation at 94°C for 2 minutes, 35 cycles of 94°C for 30 seconds, 50°C for 1 minute, 72°C for 1.5 minutes, and a final extension of 72°C for 10 minutes to ensure complete double-stranded DNA synthesis. The primers used for the PCR amplification have been previously described and included Bunya 1 (GTCACAGTAGTGTACTCCAC) and Bunya 2 (CTGACAGTAGTGTGCTCCAC), which amplifies the S segment, M14C (CGGAATTCAGTAGTGTACTACC) and M619R (GACATATG(CT)TGATTGAAGCAAGCATG) that amplifies the M segment, and M13CBunL1C (TGTAAAACGACGGCCAGTAGTGTACTCCT) and BunL605R (AGTGAAGTCICCATGTGC), which amplifies the L segment.[24]

Sequencing and phylogenetic analyses. To genetically characterize the GROV strains isolated in Peru, partial sequences of the S, M, and L segments were obtained and compared with those of GROV isolates from Brazil, Colombia, and Bolivia using a previously described methodology.[24] Purified PCR products were sequenced directly, and sequencing analyses of the PCR products were performed using an Applied Biosystems (Foster City, CA) Prism automated DNA sequencing kit according to the manufacturer's protocol. Sequences were aligned using the Clustal program in the MacVector (MacVector Inc., Cary, NC) software package, and phylogenetic analyses were performed using the maximum parsimony, neighbor-joining, and maximum likelihood methods implemented in the phylogenetic analysis using parsimony (PAUP) software (Sinauer Associates, Sunderland, MA).[25,26] For the neighbor-joining analyses, the HKY85 distance was used. Bootstrap values to place confidence values on groupings within trees were calculated based on 1,000 replicates.

Statistical analyses. Proportions were compared using a χ^2 test using the FREQ procedure in SAS (SAS version 8; SAS Institute Inc., Cary, NC). Risk factors for infection with GROV were evaluated by logistic regression using LOGISTIC in SAS. Models were constructed with the dichotomous dependent variable: PRNT positive for GROV antibody at a titer of ≥ 20 and the following independent variables: gender, age (adult or child), occupation, type of house, travel history, and neighborhoods.

RESULTS

Description of GROV cases detected through febrile surveillance. In 1995, GROV was isolated for the first time

TABLE 2
Principal clinical manifestations for 13 patients with confirmed Guaroa virus infection

Sign and symptom	Patients (%)
Headache	92
Malaise	92
Chills	85
Myalgia	77
Arthralgia	69
Bone pain	62
Retro-orbital pain	54
Nausea	46
Asthenia	46
Abdominal pain	23
Conjunctival injection	23
Vomiting	23
Rash	15
Rhinorrhea	15
Cough	15
Weight Loss	8
Petechiae	8
Ear pain	8
Arthritis	8
Expectoration	8

in Peru from a patient presenting with an undifferentiated febrile illness. Between 2000 and 2009, 16 additional confirmed GROV cases and 30 presumptive cases were identified in patients presenting with febrile illness. The most common symptoms among these patients were headache, chills, malaise, myalgia, arthralgia, and bone pain (Table 2). The majority of patients with confirmed GROV infection were males (12 of 17, 70.6%) with a mean age of 28.5 years (range = 17–43) (Table 1). These patients were mainly miners, wood cutters, agricultural workers, and students living in areas with high levels of arbovirus circulation.[17,19–23,27–30] Fifteen of the cases were detected in Peru, whereas two GROV cases were confirmed in Cochabamba, Bolivia, providing the first evidence of circulation in that country. The Bolivian patients were a 17-year-old female student and a 24-year-old male agricultural worker. Year-round GROV activity was observed in Peru.

The largest number of GROV cases were detected in 2007 and 2008 from samples collected in La Merced, Junín, and Puerto Maldonado, Madre de Dios. One GROV case was detected in the coastal city of Tumbes in Peru in 2007 (Tables 1 and 3). A slight increase in GROV activity was observed in 2007 in La Merced and in 2008 in Madre de Dios (Table 3). The majority of patients with GROV infection (44% of the cases) in Puerto Maldonado reported having recently visited or worked in Bajo Puquiri, a gold-prospecting and extraction area in Madre de Dios Department that is currently undergoing intensive environmental modification.

Risk factors for GROV infection in the city of Iquitos, Peru. Antibody prevalence and risk-factor data were obtained from blood samples collected as part of a cross-sectional antibody prevalence study carried out in four neighborhoods in Iquitos after an outbreak of febrile illness associated with VEEV infection.[18] Iquitos was selected for study, because GROV was first detected in this city in 1995 and thus, there was evidence that the virus had been circulating in the area for more than 10 years.

In Iquitos, the overall antibody prevalence of GROV was 13% (144/1,124). The prevalence of GROV antibodies in the Iquitos population increased with age after adulthood (> 19 years of age), suggesting endemic circulation of the virus in this Amazon region of Peru (4.4% in 5- to 9-year-olds to 35.9% in 60- to 69-year-olds) (Figure 2). The antibody prevalence in adults was 16% compared with 5.6% in children [odds ratio (OR) = 3.3; 95% confidence interval (CI) = 1.992–5.467]. Persons who reported overnight travel had higher antibody prevalence rates than those who did not ($P < 0.05$).

Persons with high-risk occupations were fishermen, wood cutters, and oil workers; these groups had a higher prevalence of GROV antibodies than people with other occupations [7/23 (30%) versus 137/1,101 (12%)]. In addition, persons living in concrete/brick houses had significantly lower antibody prevalence rates than those living in wood houses (OR = 0.312; 95% CI = 0.179–0.546), and persons living in neighborhoods closer to the rivers surrounding the city (Belen, Bellavista, and San Juan) also had a higher antibody prevalence than those in the north-central parts of Iquitos where socio-economic conditions are higher (Figure 3). The univariate logistic regression analysis did not detect an association between gender and GROV antibody prevalence.

Genetic characterization of the GROV isolates from Peru and other regions of South America. Phylogenetic analyses using maximum parsimony, neighbor-joining, and maximum likelihood methods all generated similar tree topologies. Only the neighbor-joining phylogenetic trees are shown for simplicity reasons. The neighbor-joining tree based on the partial S and L segment sequences revealed a single genotype within the isolates from Peru and Bolivia. In contrast, strains from Colombia and Brazil (isolated between 1956 and 1964) differed by 4% at the amino acid level compared with the more recent (1995–2008) Peruvian and Bolivian isolates, and thus, they group within different genotypes in the phylogenetic tree (Figures 4 and 5). The neighbor-joining tree based on the

TABLE 3
Cases of Guaroa virus infection among febrile patients residing in Peru and Bolivia

	Madre de Dios			Junin				Iquitos, Loreto							Tumbes		Cusco	Yurimaguas		Bolivia		
Guaroa virus cases	2007	2008	2009	2006	2007	2008	2009	1995	1999	2004	2005	2007	2008	2009	2007	2008	2008	2007	2009	2007	2009	Total
Virus isolations	4	2	0	0	3	0	0	1	1	1	0	0	0	0	0	0	0	0	0	1	1	14
Seroconversion	1	0	0	1	0	0	0	ND	ND	ND	0	0	0	0	1	0	0	0	0	0	0	3
Presumptive cases	3	8	2	0	3	1	0	ND	ND	ND	1	2	3	2	0	0	3	1	1	0	0	30
Total confirmed and presumptive GROV cases	8	10	2	1	6	1	0	1	1	1	1	2	3	2	1	0	3	1	1	1	2	50
Total number febrile cases seen at site	593	301	355	120	105	133	111	867	810	2,308	1,459	1,197	1,885	1,104	193	264	121	309	280	645	537	13,697
Percentage GROV cases/ total febrile cases	1.3	3.3	0.6	0.8	5.7	0.8	0	0.1	0.1	0.04	0.07	0.2	0.2	0.2	0.5	0	2.5	0.3	0.4	0.2	0.4	0.37

ND = not done.

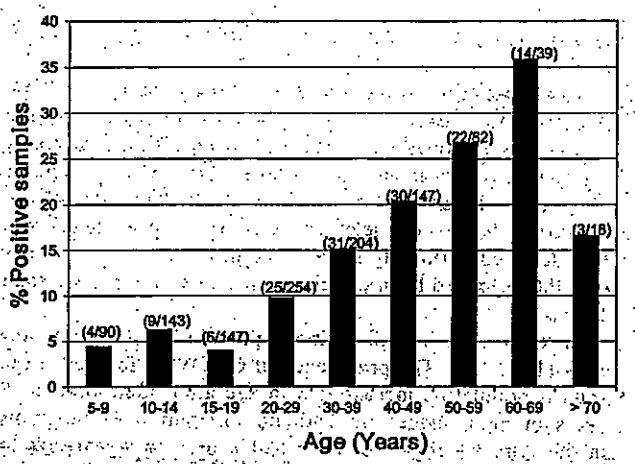

FIGURE 2. Guaroa virus antibody prevalence among residents by age groups in Iquitos.

M segment produced a similar tree topology as the S and L phylogenetic tree; however, the strains isolated from Junin, Peru grouped within a distinct genotype from the other isolates from different geographical regions in Peru (Figure 6).

DISCUSSION

GROV was initially isolated in Colombia from asymptomatic individuals as well as from persons exhibiting mild fever, raising the question of whether the virus consistently causes disease.[1] Later, GROV cases identified in Brazil included individuals with fever and other symptoms, such as headache, myalgia, and prostration; however, several of these subjects also had *Plasmodium falciparum* infections.[31,32] GROV was also isolated from the liver biopsy of a Brazilian patient with paralysis.[2] In the present study of febrile illnesses in Peru and Bolivia, additional evidence for the disease potential of GROV was obtained. Seventeen patients who presented with undifferentiated febrile illnesses were diagnosed with GROV infection.

The most common clinical symptoms in the patients with confirmed GROV infection were chills, malaise, bone pain, headache, retro-orbital pain, myalgia, and arthralgia. Given the non-specific clinical manifestations in these cases, it would be very difficult to differentiate GROV infection clinically from other endemic arboviral illnesses. Because our surveillance activities included only eight sites in Peru and three in Bolivia (Figure 1), it is very likely that GROV infections occur in other areas of these countries but remain undiagnosed. Additional studies are needed to determine the ratio of apparent to unapparent cases and to fully measure the burden and the public-health impact of GROV infection in Peru, Bolivia, and other regions of South America.

Previous epidemiologic studies conducted in South America revealed a high antibody prevalence to GROV in selected

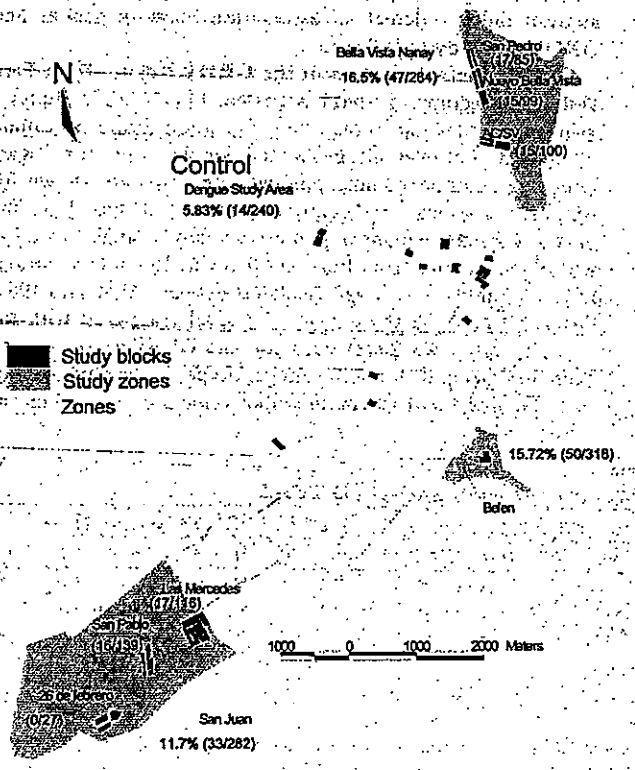

FIGURE 3. Guaroa virus antibody prevalence among residents by neighborhood in Iquitos.

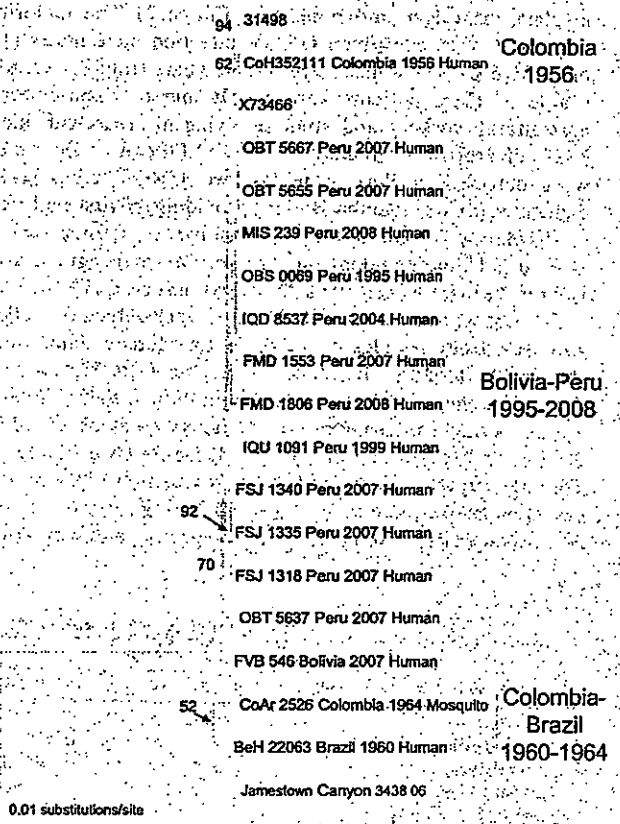

FIGURE 4. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the S segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Viruses are labeled by code designation, country name, year of isolation, and source. Numbers indicate bootstrap values.

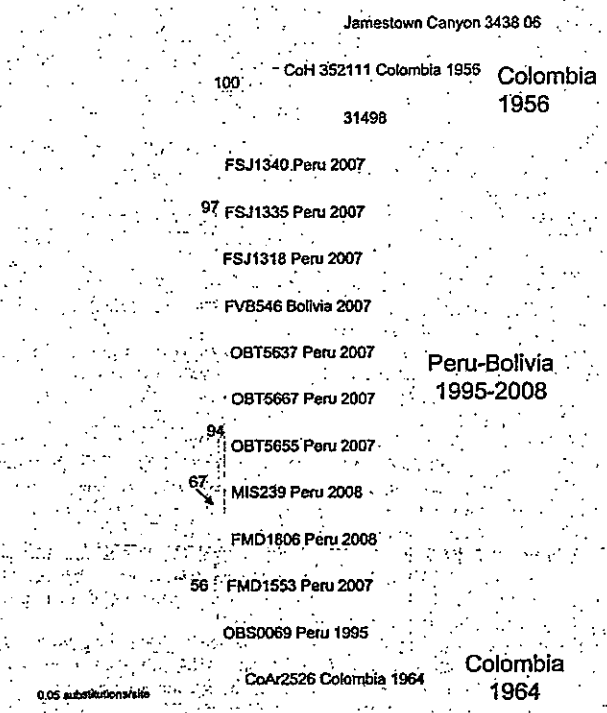

FIGURE 5. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the M segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Viruses are labeled by code designation, country name, and year of isolation. Numbers indicate bootstrap values.

populations in Colombia (43%) and Brazil (18%).[1,31] Low HI antibody titers to GROV antigen were also found in sera of residents from Argentina, Peru, and Guatemala.[9] However, further testing of the positive samples, using a confirmatory PRNT to GROV, was not performed. Because of the cross-reactivity of HI antibodies among orthobunyaviruses, it is uncertain whether those antibodies were specific to GROV or to other related members of this genus that are known to circulate in the area.[31,33]

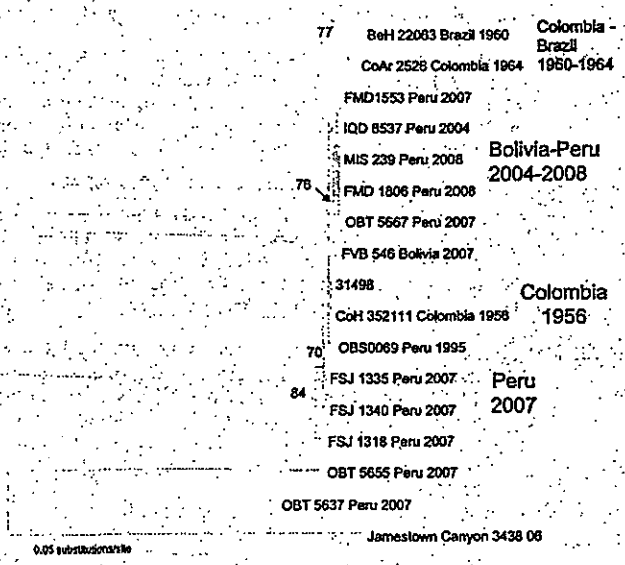

FIGURE 6. Neighbor-joining phylogenetic tree for Guaroa virus generated based on partial sequences of the L segment. The tree was rooted using Jamestown Canyon virus as the outgroup. Numbers indicate bootstrap values.

To further investigate the frequency of GROV infection in Peru and the risk factors associated with infection in an endemic area of transmission, human serum samples collected in Iquitos in 2006 were assayed for GROV antibodies. An overall antibody prevalence rate of 13% was found among the Iquitos population. These results are consistent with previous serological studies done in Peru in 1965.[29] Factors associated with infection in Iquitos included living in neighborhoods located near surrounding rivers that are seasonally flooded and occupations such as agriculture, fishing, and mineral prospecting and extraction. Results from our study also suggested that GROV transmission probably occurs in the forest or away from home.

Although GROV was first detected in Iquitos in 1995, the number of confirmed infections identified in our current surveillance activity was relatively low (~0.04–0.2%), despite the 13% overall antibody prevalence rate. In contrast, the presence of GROV in Madre de Dios was not detected until 2007, when evidence of GROV infection was observed in about 1% of the febrile cases in the region. By 2008, 3% of the febrile cases at the site had evidence of recent GROV infection. Eight of eighteen patients with evidence of GROV infection (2007–2008) reported recent work or travel in the area. It seems likely that environmental and land-use changes as well as human migration have played an important role in the emergence of this human pathogen in Madre de Dios. Improper and unregulated mining activities have been implicated as a factor responsible for the emergence of other vector-borne diseases such as malaria and yellow fever in Brazil.[34] Further research is needed to determine more precisely the impact of these activities on the emergence of GROV and other arboviral diseases in Madre de Dios.

Despite our current knowledge of GROV as a human pathogen, little is known about its reservoir hosts and vectors, especially in Peru. Earlier investigations in Colombia, Panama, and Brazil detected the virus in *Anopheles* mosquitoes[4,31]; however, studies to evaluate the susceptibility and transmission potential of this mosquito genus have not been done. During previous ecological studies conducted in the Amazon region of Peru, many arboviruses were isolated from other mosquito genera,[23] but GROV was never isolated from these other mosquitoes, suggesting that they are not involved in GROV transmission. Additional ecological studies are needed in Peru and Bolivia to identify the mosquito vectors and reservoir hosts involved in GROV transmission. Likewise, previous serological investigations in Brazil suggested that birds may act as reservoir hosts for GROV; however, these results remain to be confirmed.[9]

In summary, results of this study further confirm that GROV is a cause of febrile illness among humans in tropical regions of Central and South America.

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別紙様式第2-1

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	総合機構処理欄
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一般的名称	人赤血球濃厚液	研究報告の公表状況	Stone R. Science. 2010 Oct 1;330(6000):20-1.	公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○感染症:中国中央部における新型致死性ウイルスの特定
中国中央部でこの3年間、夏になると数百人が高熱と胃腸障害をきたし、多くの患者が多量出血し、ある地域では患者の30%程度が死亡した。ヒト顆粒球アナプラズマ症が疑われたが、テキサス大学医学部のダニ媒介性疾患の専門家が新型のブニヤウイルスを特定した。その後の研究によりこのウイルスは重症発熱性血小板減少症候群(SFTS)ウイルスと命名され、ブニヤウイルス科フレボウイルス属に分類された。しかしこのウイルスの感染による致死率や、媒介生物はまだ分かっていない。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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
中国で新型で致死性のブニヤウイルスが特定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受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を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。

Science 1 October 2010:
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NEWS OF THE WEEK

INFECTIOUS DISEASES:

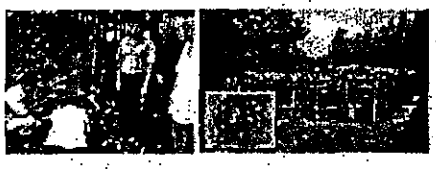
Rival Teams Identify a Virus Behind Deaths in Central China

Richard Stone

BEIJING—When Xue-jie Yu came to China last year to probe a lethal fever outbreak, everyone—Yu included—assumed he would provide damning testimony against a known suspect. Every summer for 3 years, hundreds of people in central China came down with an illness characterized by high fever and gastrointestinal (GI) distress. Many victims bled profusely, and an alarming number of the sick—rough estimates are as high as 30% in some areas—died. By early 2007, scientists at the Chinese Center for Disease Control and Prevention (CDC) here fingered the killer as human granulocytic anaplasmosis (HGA), an emerging bacterial infection from tick bites. But to Yu, an expert on tick-borne diseases at the University of Texas Medical Branch in Galveston, things didn't add up.

"The fatality rate was too high," Yu says, and in his experience it was "rare" for HGA patients to have GI symptoms. Working at the Chinese CDC's National Institute of Communicable Disease Control and Prevention (NICDC) here, Yu tested blood samples for *Anaplasma phagocytophilum*, the HGA bacterium—and came up empty. Last December, his team identified a new kind of bunyavirus, a family that includes infamous members such as hantavirus and Rift Valley fever virus. The finding, in a paper submitted to *The New England Journal of Medicine* (*NEJM*), unmasks a dangerous new emerging virus—not a bacterial outbreak—and explains why antibiotics failed to stop it.

Behind the scenes, however, a fierce argument has broken out over who discovered the virus. This summer, a Chinese CDC team led by hantavirus expert Li Dexin, director of the agency's Institute of Virology, also uncovered a bunyavirus—possibly the same one Yu's group identified. They have submitted a deeper analysis of the pathogen, including complete RNA sequences of 11 strains, to *The Lancet*. Yu charges Li's group with trying to rob him of the discovery; Li says Yu's viral sequence is incomplete and that his team identified the virus as the killer.



Into the hot zone. Xue-jie Yu (*left*, in blue shirt) looks on as farmers check a dog for ticks; forest-hugging farms (*right*) were hard hit by the emerging virus (*inset*).

CREDITS: COURTESY OF XUE-JIE YU/ UNIVERSITY OF TEXAS MEDICAL BRANCH; (INSET) COURTESY OF LI DEXIN/CHINESE CDC

[Larger version of this image]

Several key questions are disputed or unanswered. For starters, researchers do not know how lethal the virus is. The mortality rate may be high in China in part because clinics often prescribe the steroid dexamethasone to bring down high fevers; steroids suppress the immune system, which usually worsens infections. Scientists also differ on whether the virus should be handled in a biosafety level 3 facility—reserved for dangerous pathogens—or in less secure laboratories. And although the infection shows a seasonal pattern associated with tick-borne diseases—cases begin in early spring and peak in midsummer before tapering off by autumn—the vector is still a mystery.

One indisputable fact is that the emerging disease has claimed scores of lives—mostly of farmers—in China's heartland. The first documented outbreak was in 2006, in Anhui Province. At that time, a team led by NICDC Director Xu Jianguo, Chinese CDC's chief bacteriologist, rushed to Anhui. Using PCR they found *A. phagocytophilum* DNA in the blood of one patient who died and in family members and hospital staff who became infected. HGA had been recognized in the United States in 1990 and in Europe in 1997; Xu's group reported China's first cases in the 19 November 2008 issue of *The Journal of the American Medical Association*.

Curiously, however, none of the patients recalled having been bitten by ticks. And when outbreaks recurred in 2007 and 2008, the disease did not respond to antibiotics. Thinking it might have an unusual *Anaplasma* variant on its hands, NICDC in 2009 invited Yu as a short-term visiting researcher under the 1000 Talents Program, which brings overseas scientists to China.

That summer, the pathogen surfaced in Henan and Hubei provinces. In June, Yu went to Hubei's capital, Wuhan, to collect blood samples from patients. "They did not look like typical HGA cases," he says. After he failed to detect *A. phagocytophilum*, Yu says he urged Chinese CDC scientists to consider a viral pathogen—but researchers there flatly rejected the idea. Yu persisted and spotted virus particles that December in cell culture using electron microscopy. Then in February, he says, a member of his Texas lab, Yan Liu, "cracked the code of the viral genome." Two days after he informed scientists at the Chinese CDC about his findings, he says, his 1000 Talents affiliation with NICDC was terminated.

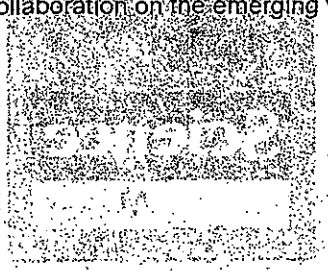
Chinese CDC Director Wang Yu was intrigued by Xue-jie Yu's findings and invited him to share them at a 15 April meeting at CDC headquarters to plot strategy for studying the disease. Among the attendees were Li and CDC virologist Liang Mifang; they found Yu's presentation unconvincing. "He said he isolated a bunyavirus, but he had gotten just fragments," says Liang. Yu confirms that the virologists were dismissive: "Li tried to deny the importance of my work," Yu says. Yu and his colleagues have named the virus Dabie Mountain virus after the range that straddles the borders of Hubei, Anhui, and Henan provinces where they collected samples. But Yu was not invited back to China this summer

to continue his research. "I am the first scientist to discover the viral pathogen for an emerging infectious disease who has no access to study the virus and the disease anymore," he says.

In May, the Chinese CDC set up surveillance for the pathogen in Henan and Hubei provinces. The disease flared up in four other provinces as well, and Li's team collected blood and serum from all six affected provinces. They amplified viral RNA sequences and from more than 500 clones linked 14 to bunyavirus. They also isolated bunyavirus in cell culture and sequenced 11 strains. They have named it severe febrile and thrombocytopenic syndrome (SFTS) virus and have classified it in the phlebovirus genus of bunyavirus. Li's group also detected the virus in 35 patients from three provinces. "It's solid work. They clearly show that a new virus is causing disease," says a U.S. scientist who has seen the data and asked to remain anonymous.

But the rival claim didn't sit well with Xue-jie Yu. When he heard that Li's team had submitted its findings to *The Lancet*, he sent an e-mail to the journal accusing Li of poaching his discovery. Liang says that's not true: "All data in our manuscript belong to us, not anyone else," she says. On 17 September, *The Lancet* asked Li's group to withdraw the paper and resubmit it after settling the authorship dispute.

The squabbling has put Wang, the Chinese CDC's director, in an awkward position. There is, no doubt, he says, that Xue-jie Yu "discovered the novel bunyavirus." While noting that Yu's results are not as "rich" as Li's team's, Wang says, "everyone knows what a scientific breakthrough is, and what is accumulating work." After the *NEJM* paper is published, he hopes, "other papers can go smoothly." But it may take Wang's best diplomatic skills to get any collaboration on the emerging virus to go smoothly.



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医薬品 研究報告 調査報告書

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販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Iwanaga M, Watanabe T, Utsunomiya A, Okayama A, Uchimaru K, Koh KR, Ogata M, Kikuchi H, Sagara Y, Uozumi K, Mochizuki M, Tsukasaki K, Saburi Y, Yamamura M, Tanaka J, Moriuchi Y, Hino S, Kamihira S, Yamaguchi K; Joint Study on Predisposing Factors of ATL Development investigators. Blood. 2010 Aug 26;116(8):1211-9. Epub 2010 May 6.	日本

研究報告の概要

○無症候性ヒトT細胞白血病ウイルスⅠ型(HTLV-1)キャリアにおける日本国内の前向き調査:プロウイルス量と疾患発症との関連

無症候性HTLV-1キャリアにおける成人T細胞白血病(ATL)発症の確実なリスク因子は、現在も不明である。近年、HTLV-1プロウイルス量は、ATLの重要な予測因子として評価されているが、少数の小規模前向き試験が実施されているだけである。2002年～2008年に、登録された無症候性HTLV-1キャリア1,218名(男性426名、女性792名)を前向きに評価した。登録時のプロウイルス量は、女性と比べ男性(末梢血単核細胞[PBMCs]100あたりの中央値1.39 vs 2.10 ; P<0.001)、40歳以下の集団に対し年齢40～49歳および50～59歳の集団(それぞれP = 0.02 , 0.007)、ATL家族歴を有さない集団に対し有する集団(PBMCs 100あたりの中央値1.33 vs 2.32 ; P=0.005)の方が、有意に多かった。14名の登録者が顕在性ATLへ進行した。この登録者の登録時のプロウイルス量は多かった(範囲:PBMCs 100あたり4.17～28.58)。登録時のプロウイルス量が4コピー以下の登録者は、ATLを発症しなかった。また多変量Cox解析では、プロウイルス量だけでなく、高年齢、ATLの家族歴、他疾患治療時における初回HTLV-1検査が、ATLの進行の独立リスク因子であることが示された。

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

日本国内で無症候性ヒトT細胞白血病ウイルスⅠ型キャリアのプロウイルス量と疾患進行の前向き調査を行ったところ、プロウイルス量は男性や40歳以上、成人T細胞白血病(ATL)の家族歴を有する集団で高く、登録時のプロウイルス量がATLの発症に影響を与えたとの報告である。

今後の対応

日本赤十字社では、献血時のスクリーニング法として、化学発光酵素免疫測定法(CLEIA)によるHTLV-1抗体のスクリーニング検査を行っている。今後も引き続き情報の収集に努める。

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Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan

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Human T-cell leukemia virus type I (HTLV-1) proviral load and disease progression in asymptomatic HTLV-1 carriers: a nationwide prospective study in Japan

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Definitive risk factors for the development of adult T-cell leukemia (ATL) among asymptomatic human T-cell leukemia virus type I (HTLV-1) carriers remain unclear. Recently, HTLV-1 proviral loads have been evaluated as important predictors of ATL, but a few small prospective studies have been conducted. We prospectively evaluated 1218 asymptomatic HTLV-1 carriers (426 males and 792 females) who were enrolled during 2002 to 2008. The proviral load at enrollment was significantly higher in males than females (median, 2.10 vs 1.39 copies/100 peripheral blood mononuclear cells [PBMCs]; $P < .001$), in those 40 to 49 and 50 to 59 years of age than that of those 40 years of age and younger ($P = .02$ and .007, respectively), and in those with a family history of ATL than those without the history (median, 2.32 vs 1.33 copies/100 PBMCs; $P = .005$). During follow-up, 14 participants progressed to overt ATL. Their baseline proviral load was high (range, 4.17-28.58 copies/100 PBMCs). None developed ATL among those with a baseline proviral load lower than approximately 4 copies. Multivariate Cox analyses indicated that not only a higher proviral load, advanced age, family history of ATL, and first opportunity for HTLV-1 testing during treatment for other diseases were independent risk factors for progression of ATL. (*Blood*. 2010;116(8): 1211-1219)

Introduction

Human T-cell leukemia virus type I (HTLV-1), the first human retrovirus to be identified, is etiologically associated with adult T-cell leukemia (ATL), HTLV-1–associated myelopathy/tropical spastic paraparesis (HAM/TSP), and HTLV-1 uveitis/HTLV-1–associated uveitis (HU/HAU).[1-3] Worldwide, endemic areas for the virus are unevenly distributed, which include southwest Japan, the Caribbean islands, South America, and a part of Central Africa.[4] In Japan, the number of HTLV-1 carriers was estimated to be approximately 1.2 million people during the late 1980s.[5] The majority of HTLV-1 carriers remain asymptomatic throughout their lives. The lifetime risks of developing ATL and HAM/TSP are estimated to be approximately 2.5% to 5%[6,7] and 0.3% to 2%,[8,9] respectively.

Several molecular biologic studies have reported that various cellular dysfunctions induced by viral genes (eg, *tax* and *HBZ*), genetic and epigenetic alterations, and the host immune system may be involved in the leukemogenesis of ATL.[10-12] Clinical and epidemiologic studies have also reported a variety of possible risk factors for ATL, including vertical transmission of HTLV-1 infection, male gender, a long latent period, increased leukocyte counts or abnormal lymphocyte counts, and higher levels of anti–HTLV-1 antibody titers and soluble interleukin-2 receptor-α.[13-19] However, there are no clear determinants that separate those who develop ATL from those who remain healthy carriers.

Recently, HTLV-1 proviral load levels have been evaluated as important predictors of development of ATL and HAM/TSP. Some cross-sectional studies showed that HTLV-1 proviral load levels were higher in ATL and HAM/TSP compared with asymptomatic HTLV-1 carriers.[20,21] However, the proviral load levels of asymptomatic HTLV-1 carriers exhibited a very wide range,[20,22,23] and these levels may vary by sex, race, habitats, and comorbidities.[24] The proviral load levels of asymptomatic HTLV-1 carriers were also examined serially in some prospective studies; however, the

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number of reported cases was very small.[25-28] Although these previous studies suggest a possible important role for HTLV-1 proviral load in the development of ATL and HAM/TSP, the association between HTLV-1 proviral load and diseases development remains unclear.

The identification of risk factors for developing ATL among virus carriers is necessary to prevent these diseases in HTLV-1 endemic areas. To investigate detailed viral- and host-specific determinants of disease development, larger and longer prospective studies are warranted. In 2002, we established a nationwide cohort study for asymptomatic HTLV-1 carriers in Japan named the Joint Study on Predisposing Factors of ATL Development (JSPFAD).[29] The main objective of this project is to establish reliable predisposing factors for developing ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. Here, for the first-time, we report the study method, baseline demographic characteristics, and distribution characteristics of baseline HTLV-1 proviral load of asymptomatic HTLV-1 carriers. We have also evaluated progression to ATL and its risk predictors.

Methods

Participants and study design

The JSPFAD is a nationwide prospective study of HTLV-1 carriers, which was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. The project was established in August 2002 by Japanese clinicians and basic researchers of 41 institutions composed of 14 university hospitals and 27 educational hospitals located in various areas of Japan (supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Objectives of the project are to establish reliable predisposing factors for development of ATL by prospectively following a large number of asymptomatic HTLV-1 carriers. This includes performing clinical examinations and biomarker assays, as well as establishing a biomaterial resource bank of plasma, viable peripheral blood mononuclear cells (PBMCs), frozen PBMCs pellets, and genomic DNA from PBMCs of HTLV-1–infected persons for the future evaluations with new molecular biology techniques.

Hematologists at the collaborating institutions were responsible for enrolling participants after receiving approval from their Institutional Review Boards. The study protocol was approved by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Eligible participants were those who had known of their HTLV-1 infection and had confirmed the HTLV-1–positive serology at any of the medical institutions. Potential participants visited any of the collaborating institutions directly or via the website of the JSPFAD (www.htlv1.org/). They received adequate explanations for the enrollment procedure from the hematologists at the collaborating institutions. Enrollment was conditional on participants giving written informed consent in accordance with the Declaration of Helsinki. The primary participants were asymptomatic HTLV-1 carriers. A small number of patients with definite ATL, HAM/TSP, and HU/HAU were also enrolled as controls.

Data collection and sample storage

After providing written informed consent, participants were expected to fill out a questionnaire regarding demographic information, to provide peripheral blood samples, and to periodically visit the institution for follow-up. After reconfirming the asymptomatic HTLV-1 carrier status of the participants, hematologists at the collaborating institutions assigned a unique identification number to each participant and subsequently sent all materials (individual questionnaire sheets, clinical data, and blood samples drawn into ethylenediaminetetraacetic acid and heparin tubes) to the JSPFAD office (Department of Medical Genome Sciences, Laboratory of Tumor Cell Biology, Graduate School of Frontier Sciences, University of Tokyo, Japan).

The self-administered questionnaire included items on demographic characteristics, birthplaces of the participants and their mothers, family history regarding HTLV-1 status and HTLV-1–associated diseases, length of marriage, partner's HTLV-1 status, first opportunity for HTLV-1 testing, and histories of disease manifestations other than HTLV-1–associated diseases. Additional questionnaire items, information on prior blood transfusion, and smoking habits (present, past, or nonsmoking) were also included after April 2008.

Clinical data included information on the date of visit, complete blood cell count, differential cell counts (including abnormal lymphocytes per 100 leukocytes), lactate dehydrogenase, HTLV-1 serologic test, comorbidities other than HTLV-1–associated diseases, and the development of any HTLV-1–associated diseases during follow-up. Blood samples were collected at enrollment, annually thereafter (in principal), and as needed. Blood samples sent to the study office at the University of Tokyo were separated into plasma, PBMCs, and genomic DNA and then used for viral marker assays at the University of Tokyo or stored for the biomaterial bank at the Japanese Red Cross Fukuoka Blood Center.

Viral marker assays

HTLV-1 proviral load of PBMC samples was measured by real-time polymerase chain reaction (PCR) using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems Japan), as previously described with minor modifications.[30,31] Genomic DNA from PBMCs was isolated using a QIAGEN Blood Kit (QIAGEN). Quantitative real-time PCR was performed using multiplex PCR with 2 sets of primers specific for the HTLV-1 provirus and the human gene encoding the RNase P enzyme. The primers and the probe for the gene encoding RNase P were purchased from Applied Biosystems; those for the pX region of the HTLV-1 provirus were described previously.[30,31] Genomic DNA of normal control PBMCs mixed with a plasmid DNA, which contained almost the whole genome of the HTLV-1 provirus (*SacI* site of 5′-LTR to *SacI* site of 3′-LTR), was used as control template. The copy number of the plasmid DNA was calculated based on the size and weight of the plasmid DNA, as measured by spectrophotometry. The proviral loads were expressed as copy numbers per 100 PBMCs, based on the assumption that infected cells harbored 1 copy of the integrated HTLV-1 provirus per cell. Samples with a higher proviral load (> 20 copies/100 PBMCs) were subjected to Southern blot analysis to examine the clonality of the infected cells. Assays to detect the integrated band of HTLV-1 provirus genome were described previously.[32] Genomic DNA samples (10 mg) were digested with *PstI* or *Eco*RI restriction enzymes and were size-fractionated on 0.7% agarose gels. They were then transferred onto a nylon membrane by the Southern blot technique. Hybridization to randomly primed ^{32}P-labeled DNA probes for the whole proviral genome (*SacI* to *SacI* fragment of the HTLV-1 proviral genome) was performed, followed by appropriate stringency washing steps and autoradiography. Soluble interleukin-2 receptor was measured by a commercial laboratory (SRL Inc) using an enzyme-linked immunosorbent assay (Endogen) and reported as units per milliliter.

Statistical analysis

Analyses were performed for participants who enrolled as of December 2008. Age at enrollment was categorized into 5 groups: younger than 40, 40 to 49, 50 to 59, 60 to 69, and 70 years or older. Geographic location was divided into 4 areas: northern (Hokkaido and Tohoku), metropolitan (Tokyo, Osaka, and Nagoya), southern (Kyushu and Okinawa), and others (supplemental Figure 1). First opportunity for HTLV-1 testing was divided into 3 categories: by screening for HTLV-1 (regional-mass, multiphasic, blood donor, and maternal screenings), by the presence of HTLV-1–infected family members (including spouse), and by the patient status under treatment for diseases unrelated to HTLV-1. A positive family history was considered to be present when participants had information on first-degree relatives (parents, siblings, or offspring) who were HTLV-1 carriers or had HTLV-1–associated diseases (ie, ATL, HAM/TSP, and HU/HAU). Any leukemia and/or lymphoma other than ATL were also taken into consideration. A positive comorbidity at enrollment was considered to be present when any information on diseases other than HTLV-1–associated diseases

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was available at enrollment. HTLV-1 proviral loads (copy numbers/100 PBMCs) were used as a continuous variable (raw and the power-transformed data) or by categorizing them into quartiles. We applied a square-root transformation to the raw data of proviral loads to reduce the skewness. Continuous data were presented as median (range) values and compared using a Mann-Whitney test. Categorical data were compared using a χ^2 test or Fisher exact test. We calculated person-years of follow-up for each participant from the date of enrollment to the date of ATL diagnosis, the date of last follow-up, or September 30, 2009, whichever came first. Cumulative progression to ATL was estimated using Kaplan-Meier curves. To estimate the effect of baseline HTLV-1 proviral load and selected demographic factors on ATL development, we performed Cox proportional hazards analyses, and expressed as hazard ratios (HR) and 95% confidence intervals (CI), which were calculated by robust sandwich variance estimates. To check for possible incompleteness in the multivariate model, we also performed analyses using sub-datasets. All statistical analyses were performed using SAS Version 9.1 (SAS Institute Japan) with a 2-tailed significance level of .05.

Results

Baseline demographic characteristics

From August 2002 to December 2008, 1259 participants of asymptomatic HTLV-1 carriers were enrolled in this study. However, HTLV-1 proviral load was not measured for 41 participants. Thus, a total of 1218 participants (426 males and 792 females) were included in this analysis. Demographic characteristics of the participants at enrollment are shown in Table 1. The median ages at enrollment in the cohort were 59.6 years (range, 6.9-92.8 years) for males or 58.3 years (range, 17.8-90.3 years) for females. The largest percentage of study participants was from the southern area, which is a well-known HTLV-1 endemic area in Japan, followed by the metropolitan area. The southern area also had the largest percentage for birthplaces for most participants and their mothers.

One-half of the participants came to know of their HTLV-1 infections through screening for HTLV-1, and one-fourth was informed of their infections while receiving treatments for diseases other than HTLV-1–associated diseases. More than half of the participants did not know their family status of HTLV-1 infection. Only 119 female participants knew about the HTLV-1 infection status of their husbands, of whom 53 (45%) of the husbands were positive for HTLV-1 (data not shown). However, we were not able to obtain reliable information on male-to-female transmission for the female participants. We obtained information on comorbidities at enrollment from 257 participants, of which 45 had comorbid infectious diseases (eg, strongyloidiasis, chronic bronchitis, hepatitis C virus infection, lymphadenitis), 29 had autoimmune diseases (rheumatoid arthritis, chronic thyroiditis, Sjögren syndrome, and other autoimmune or chronic inflammatory diseases), 80 had a variety of definite malignant diseases other than ATL (non-Hodgkin lymphoma, acute myeloid leukemia, gastric cancer, lung cancer, or other malignancies), 16 had skin diseases, and 87 had other common diseases (eg, hypertension, diabetes).

Distributions of baseline HTLV-1 proviral load

Figure 1 shows distribution of baseline HTLV-1 proviral load in 1218 participants. There was a wide range of skewness in the raw data, with a median of 1.60 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs; 25th-75th percentile, 0.29-4.54 copies/100 PBMCs; Figure 1A). The square-root transformation reduced the skew in the raw data, with a median of 1.26 copies/100

Table 1. Baseline demographic characteristics of asymptomatic HTLV-1 carriers

Variable	Male, no. (%)	Female, no. (%)
Total	426	792
Age, y		
Younger than 40	48 (11.3)	119 (15.0)
40-49	70 (16.4)	130 (16.4)
50-59	59 (13.8)	174 (22.0)
60-69	88 (20.7)	172 (21.7)
70 or older	121 (28.4)	197 (24.9)
Place of enrollment		
Northern area	10 (2.3)	32 (4.0)
Metropolitan area	75 (17.6)	144 (18.1)
Southern area	333 (78.2)	597 (75.4)
Other areas	8 (1.9)	19 (2.4)
Birthplace of participants		
Northern area	18 (4.2)	33 (4.2)
Metropolitan area	30 (7.0)	80 (10.1)
Southern area	240 (56.3)	400 (50.5)
Other areas	20 (4.7)	54 (6.8)
Unknown	118 (27.7)	225 (28.4)
Birthplace of participants' mothers		
Northern area	16 (3.8)	32 (4.0)
Metropolitan area	13 (3.1)	36 (4.5)
Southern area	247 (58.0)	426 (53.8)
Other areas	28 (6.6)	64 (8.1)
Unknown	122 (28.6)	231 (29.2)
First opportunity for HTLV-1 testing		
Screening for HTLV-1	209 (49.1)	452 (57.1)
Regional mass screening	77	164
Multiphasic screening	24	44
Blood donor screening	108	128
Maternal screening	0	116
Revelation of HTLV-1–positive family	35 (7.7)	101 (12.7)
During treatment of other diseases	117 (27.5)	148 (18.7)
Unknown	67 (15.7)	91 (11.5)
Family history of HTLV-1–associated diseases*		
Absent	98 (23.0)	154 (19.5)
Absent for a first-degree relative but having an		
Infected spouse	6 (1.4)	23 (2.9)
Carrier only	27 (6.3)	74 (9.3)
HU/HAU only	2 (0.5)	1 (0.1)
HAM	2 (0.5)	7 (0.9)
ATL	34 (8.0)	74 (9.3)
Leukemia or lymphoma	9 (2.1)	26 (3.3)
Unknown family history	248 (58.2)	433 (54.7)
Comorbidity†		
Absent	331 (77.7)	630 (79.5)
Present	95 (22.3)	162 (20.5)
Infectious diseases	20	25
Autoimmune diseases	3	26
Malignant diseases	36	44
Skin diseases	5	9
Other disease	28	59

HTLV-1 indicates human T-cell leukemia virus type 1; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; and ATL, adult T-cell leukemia.

*Family history was restricted to a first-degree relative. "Present" indicates that participants have a parent, sibling, or offspring diagnosed with HTLV-1-associated diseases. Family members with HAM and HU/HAU were included into the category of "HAM." Family members with ATL and HAM and/or HU/HAU were included into the category of "ATL."

†Comorbidity indicates that participants have any diseases other than HTLV-1–associated diseases at enrollment.

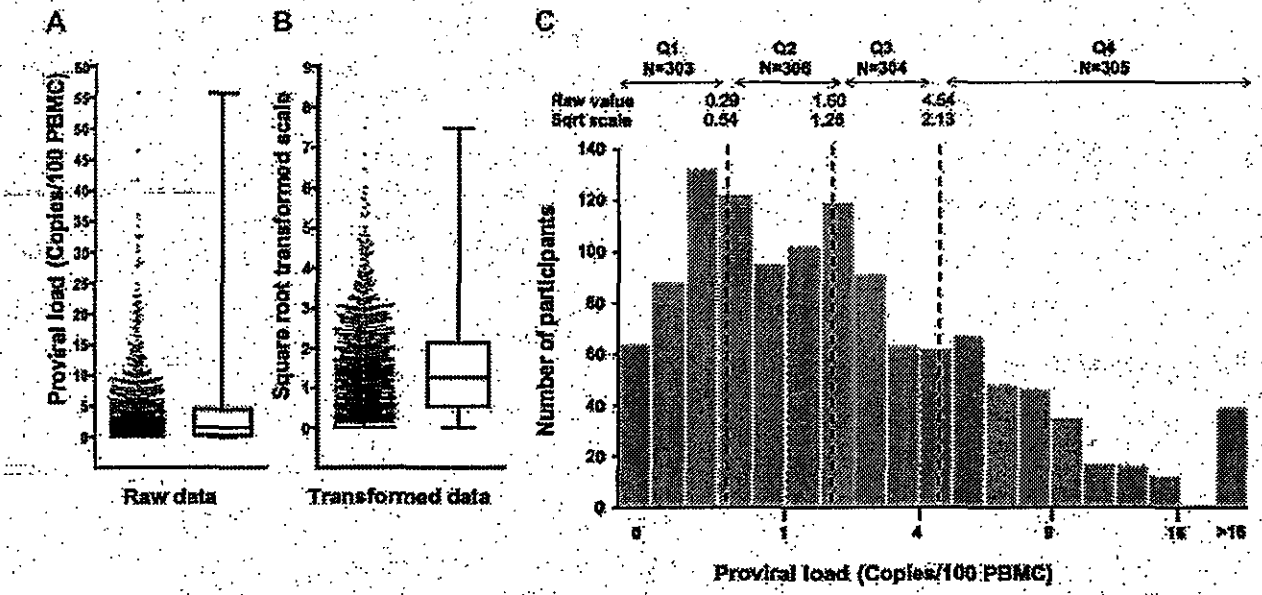

Figure 1. Distribution of baseline HTLV-1 proviral load levels among 1218 asymptomatic HTLV-1 carriers. (A) Scatter plot of raw data of proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.29 copies/100 peripheral blood mononuclear cells [PBMCs]), median (1.60 copies/100 PBMCs), and 75th percentiles (4.54 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (55.8 copies/100 PBMCs). (B) Scatter plot of square-root transformed values of the raw proviral load (left) and the vertical box and whiskers plot (right): the box delineates 25th percentile (0.54 copies/100 PBMCs), median (1.26 copies/100 PBMCs), and 75th percentiles (2.13 copies/100 PBMCs), and the whiskers delineate the minimum (0 copies/100 PBMCs) and maximum (7.47 copies/100 PBMCs). (C) The frequency of participants in the quartile distributions of proviral load. Q1 indicates quartile 1 (< 25th percentile); Q2, quartile 2 (25th percentile to median); Q3, quartile 3 (median to 75th percentile); Q4: quartile 4 (> 75th percentile); Sqrt, square-root transformation; and N, number of participants.

PBMCs (range, 0-7.47 copies/100 PBMCs; 25th-75th percentile, 0.54-2.13 copies/100 PBMCs; Figure 1B). Figure 1C shows the frequency of participants in each quartile of proviral load.

The median proviral load and a frequency of subjects in each quartile of proviral load by demographic characteristics are shown in Table 2. Males and females were significantly different in proviral load levels, with a median value of 2.10 copies/100 PBMCs (range, 0-46.6 copies/100 PBMCs) for males and that of 1.39 copies/100 PBMCs (range, 0-55.8 copies/100 PBMCs) for females ($P < .001$). Males were probably distributed in the highest quartile of proviral load level than females.

Among age groups, the median proviral load of those 40 to 49 and 50 to 59 years of age was significantly higher than that of those less than or equal to 40 years ($P = .02$ and $P = .007$, respectively). Both age groups were probably distributed in the highest quartile of proviral load levels. Because we found a significantly different median proviral load by sex, we additionally evaluated the proviral load level by age group in each sex. The highest median value was found in those 50 to 59 years of age (2.89 copies/100 PBMCs) in males, but in 40 to 49 years of age (1.49 copies/100 PBMCs) in females, although there were no statistical differences by age group for both sexes (data not shown).

Among the categories for the first opportunity for HTLV-1 testing, the proviral load level was significantly higher ($P = .002$) in participants informed of their infection during treatment for diseases unrelated to HTLV-1 compared with those who came to know of their infection by screenings (Table 2). Participants informed of their infection during treatment for diseases unrelated to HTLV-1 were probably distributed in the highest quartile of proviral load levels. There was no difference in the proviral load level between those who came to know of their infection by the presence of HTLV-1–positive family members and those who came to know of their infection by screenings.

When we evaluated the proviral load level by family history status, participants who had no family history of HTLV-1 infection, who had only HTLV-1 carriers in the family, who had only an HTLV-1 carrier husband, and who had only HU/HAU in the family were grouped together as a reference category. The proviral load levels of those with a family history of HAM/TSP (median 3.85 copies/100 PBMCs) and ATL (median 2.32 copies/100 PBMCs) were significantly higher ($P = .01$ and $P = .005$, respectively) compared with those of the reference group (Table 2). Indeed, those with a family history of HAM/TSP and ATL were probably distributed in the third and fourth quartiles of proviral load levels. Of interest, the median proviral load level of those with a family history of leukemia or lymphoma was also significantly higher ($P = .009$) compared with those of the reference group.

Among the categories for comorbidity, there was no statistical difference in the proviral load levels when we simply compared between those with and without comorbidity at enrollment (data not shown). However, when we compared those without comorbidity and those with infectious diseases at enrollment, the median proviral load of the latter was significantly higher than that of the former ($P = .05$; Table 2).

Prognosis

During a median follow-up period of 1.0 year (range, 0-6.6 years) and a total of 1981.2 person-years, 14 (1.1%) participants (4 males and 10 females) progressed to overt ATL (2 acute, 2 lymphoma, and 10 smoldering types; Table 3). The incidence rate of ATL was 7.1 per 1000 person-years for all types of ATL and 2.0 per 1000 person-years for the aggressive types (acute and lymphoma) of ATL. The median duration from date of enrollment to date of diagnosis of ATL was 13.8 months (range, 2.8-64.4 months). The cumulative probability of progression to ATL was reached 4.8% (95% CI, 1.9%-11.8%) at 5.4 years (Figure 2).

Table 2. HTLV-1 VL levels by demographic characteristics

Demographic characteristics	No.	Median VL (range) (copies/100 PBMCs)	Frequency of subjects by VL level, n (% of row) Quartile 1 (VL: < 0.29)‡	Quartile 2 (VL: 0.29-1.60)	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total	1218	1.60 (0-55.8)	603	306	304	305
Sex						
Male	426	2.19 (0-46.6)†	84 (19.7)	100 (23.5)	93 (21.8)	149 (35.0)
Female	792	1.39 (0-55.8)†	219 (27.7)	206 (26.0)	211 (26.6)	156 (19.7)
Age, y						
Younger than 40	167	1.37 (0-16.4)†	49 (29.3)	43 (25.8)	50 (29.9)	25 (15.0)
40-49	200	1.77 (0-31.7)*	43 (21.5)	52 (26.0)	51 (25.5)	54 (27.0)
50-59	273	1.84 (0-36.1)*	64 (23.4)	64 (23.4)	63 (23.1)	82 (30.4)
60-69	260	1.56 (0-46.6)	66 (25.4)	66 (25.4)	61 (23.5)	67 (25.8)
70 or older	318	1.52 (0-55.8)	81 (25.5)	81 (25.5)	79 (24.8)	77 (24.2)
First opportunity for HTLV-1 testing						
Screening	661	1.46 (0-55.8)†	182 (27.5)	160 (24.2)	175 (26.5)	144 (21.8)
Revelation of HTLV-1–positive family	134	1.45 (0-46.6)	31 (23.1)	40 (29.9)	39 (29.1)	24 (17.9)
During treatment for other diseases	265	1.93 (0-41.7)*	56 (21.1)	66 (24.9)	57 (21.5)	86 (32.5)
Unknown	158	2.08 (0-30.3)*	34 (21.5)	40 (25.3)	33 (20.9)	51 (32.3)
Family history of HTLV-1–related diseases						
Absence or carrier/HU/HAU only	385	1.33 (0-32.4)†	100 (26.0)	105 (27.2)	100 (26.0)	80 (20.8)
HAM/TSP	9	3.85 (1.2-9.4)*	0	1 (11.1)	5 (55.6)	3 (33.3)
ATL	108	2.32 (0-46.6)*	18 (16.7)	26 (24.1)	33 (30.6)	31 (28.7)
Leukemia or lymphoma	35	2.47 (0-12.8)*	3 (8.6)	9 (25.7)	11 (31.4)	12 (34.3)
Unknown family history	681	1.55 (0-55.8)	182 (26.7)	165 (24.2)	155 (22.8)	179 (26.3)
Comorbidity						
Absence	961	1.55 (0-55.8)†	241 (25.1)	234 (24.4)	244 (25.4)	242 (25.2)
Infectious diseases	45	2.75 (0-28.6)*	7 (15.6)	8 (17.8)	13 (28.9)	17 (37.8)
Autoimmune diseases	29	1.33 (0-41.7)	10 (34.5)	7 (24.1)	4 (13.8)	8 (27.6)
Malignant diseases	80	1.57 (0-19.4)	19 (23.8)	21 (26.3)	23 (28.8)	17 (21.3)
Skin diseases	16	0.60 (0.07-14.6)	6 (37.5)	5 (31.3)	3 (18.8)	2 (12.5)
Other disease	87	1.17 (0-22.0)	20 (23.0)	31 (35.6)	17 (19.5)	19 (21.8)

HTLV-1 indicates human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and ATL, adult T-cell leukemia.

*Mann-Whitney test revealed a statistically significant difference in the VL level compared with the reference group.

†Reference group.

‡The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

The median proviral load at enrollment for these 14 participants was 10.3 copies/100 PBMCs (range, 4.17-28.58 copies/100 PBMCs), which was significantly higher than those who did not develop ATL (1.56 copies/100 PBMCs; range, 0-55.8 copies/100 PBMCs; $P < .001$). Of interest, the median proviral load level at enrollment was significantly higher for those who developed smoldering types of ATL than for those who developed aggressive types of ATL (11.4 and 5.1 copies/100 PBMCs, respectively, $P = .02$), whereas the median entry age was significantly younger for the former than for the latter (59.8 and 73.9 years, respectively, $P = .02$). Distribution of the 14 participants who developed ATL by demographic characteristics and by quartile of proviral load levels is shown in Table 4. Among 14 ATLs, 13 occurred in the highest quartile of baseline proviral load (> 4.54 copies/100 PBMCs) and 1 occurred in the third quartile (1.60-4.54 copies/100 PBMCs), whereas no ATL developed in quartiles 1 and 2 (< 1.60 copies/100 PBMCs). A high frequency of ATL was also seen in older age group, those with first opportunity for HTLV-1 testing during treatment of other diseases and those with a family history of ATL. Therefore, we decided to include the baseline HTLV-1 proviral load (the square-root transformed continuous value), age, first opportunity for HTLV-1 testing, and family history into Cox hazard analyses as covariates to test the effects on the development of ATL.

We identified that baseline proviral load was strongly associated with the risk of progression to ATL on both univariate and multivariate Cox analyses. In the multivariate analysis, the adjusted HR for the square-root transformed proviral load per unit increase was 3.57 (95% CI, 2.25-5.68; Table 5). We also found that advanced age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were independently associated with the development of ATL, after adjusting the effect of proviral load. The adjusted HR for developing ATL per 5-year increase of age from 40 years was 1.67 (95% CI, 1.12-2.50). HTLV-1 carriers having a family history of ATL had 12 times higher risk of developing ATL compared with those not having the history (adjusted HR = 12.1; 95% CI, 2.26-64.7), and those who came to know their HTLV-1 infection during treatment for other diseases had 4 times higher risk of developing ATL compared with references (adjusted HR = 4.16; 95% CI, 1.37-12.6), although the CIs were wide because of the smaller group sizes (Table 5). Of interest, male gender was not a significant risk factor for developing ATL, even though the median proviral load was significantly higher in males than in females (Table 2).

Because the distribution of proviral load was skewed even after the value was square-root transformed, it was possible that ATL events in subjects with skewed high proviral loads contributed to results. To check the possibility, we performed a multivariate analysis using a sub-dataset that excluded subjects with skewed proviral load (> 16 copies in Figure 1C; n = 39, including 3 who developed ATL). Nevertheless, we observed similar results as the original dataset, although age factor was no longer statistically

Table 3. Cases who developed ATL from HTLV-I carrier status

	Demographic characteristics						Baseline clinical and biologic values					ATL development	
Case no.	Sex	Age, y	Place of birth	First opportunity for HTLV-1 testing	Family history of HTLV-1–related disease	Comorbidity at enrollment	HTLV-1 VL, copies/100 PBMCs	sIL-2R, U/mL	Abnormal lymphocytes, percentage	LDH, IU/L	WBC, $\times 10^3/mm^3$	Clinical type	Duration from enrollment, mo
Progression to aggressive type of ATL													
1	M	79.9	Southern	ATL family	ATL	None	5.47	479	2	157	4200	Acute	7.4
2	F	70.8	Southern	ATL family	ATL	None	4.73	904	0	365	9130	Acute	38.6
3	M	71.9	Southern	Other disease	None	Skin disease	4.17	1450	0	351	5140	Lymph	4.6
4	F	75.9	Southern	Unknown	Unknown	None	10.52	2090	3	308	3600	Lymph	30.6
Progression to indolent type of ATL													
5	F	60.0	Southern	Other disease	Unknown	None	9.12	340	14	192	5100	Sm	6.0
6	F	71.9	Southern	Multiphasic screening	None	None	10.60	1320	2	199	4000	Sm	29.8
7	F	59.5	Southern	Multiphasic screening	None	None	21.90	635	4.5	168	4100	Sm	12.0
8	F	74.0	Southern	Other disease	Unknown	Gallbladder cancer	10.11	1110	2	240	2700	Sm	26.8
9	F	54.1	Southern	Other disease	Unknown	None	16.95	971	2	198	5660	Sm	29.0
10	F	43.3	Southern	Pregnancy	ATL	None	13.90	372	1	ND	5400	Sm	64.4
11	F	62.2	Southern	Other disease	Unknown	Eye disease	6.06	1560	ND	508	12100	Sm	6.0
12	M	57.6	Southern	Other disease	Unknown	None	7.67	ND	2	234	5500	Sm	15.4
13	F	41.0	Metropolitan	Pregnancy	None	None	12.14	849	2.5	169	7690	Sm	12.2
14	M	66.1	Southern	Other disease	None	Prostatitis	28.58	2660	0	158	8500	Sm	2.8

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1;VL, HTLV-1 proviral load; PBMCs, peripheral blood mononuclear cells; sIL-2R, soluble interleukin-2 receptor; LDH, lactate dehydrogenase; WBC, white blood cell count; Sm, smoldering type; and ND, not done.

significant ($P = .07$; supplemental Table 1). It is also possible that effects of some of the risk factors are weighted because of only 1 patient with an event because only 14 were analyzed as events in the multivariate analyses. To check the possibility, we performed 14 leave-one-out analyses, omitting 1 of 14 cases at a time from the original dataset. The Jackknifed coefficient of each parameter revealed the stability, which indicated that none of 14 cases affected the original model (data not shown).

Discussion

Previous studies reported no significant differences in the HTLV-I proviral load by sex and age in asymptomatic HTLV-1 carriers.[21,22,24,33] In the present study, however, we found that there were significant differences in the proviral load by sex and age (Table 2). The median HTLV-1 proviral load was significantly higher in males than females. The median HTLV-1 proviral load for those 40 to 49 and 50 to 59 years of age was significantly higher than for those less than or equal to 40 years. The discrepancy between results of previous studies and those of the present study may be primarily explained by the differences in study population characteristics. We also found sex differences in age distributions of HTLV-1 proviral load; in male subjects, the median proviral load level was the highest at 50 to 59 years of age, whereas in female subjects it was highest at 40 to 49 years of age, although there were no statistical differences. These distribution characteristics of HTLV-1 proviral load are of interest when we consider the differences in sex and age at onset between ATL and HAM/TSP. ATL occurs predominantly in older males (~ 60 years), whereas HAM/TSP occurs predominantly in middle-aged females (~ 45-55 years). Thus, the proviral load levels of asymptomatic HTLV-1 carriers might be the highest in the age groups approximately 5 to 10 years before the average age at onset of ATL and HAM/TSP. These distribution characteristics may be related to differences in host immune responses to HTLV-1 and other unknown host factors.[34]

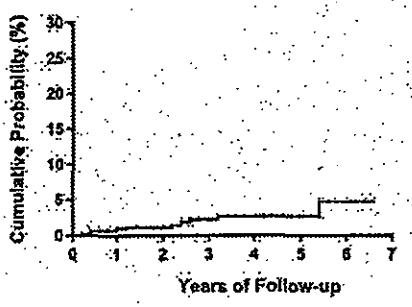

Figure 2. Probability of progression to ATL among 1218 asymptomatic HTLV-1 carriers.

The present study revealed that the median proviral load level of those with a family history of ATL or HAM/TSP was significantly higher than for those with no family history (Table 2). These results support previous studies indicating that HTLV-1–infected blood donors and asymptomatic carriers with familial HAM/TSP or ATL tend to have a higher HTLV-1 proviral load than those without family history.[21,33] In the present study, the proviral loads were also higher in those with a family history of leukemia or lymphoma than those without such history. We assume that the family history of leukemia or lymphoma may have included some ATL cases because some participants provided a diagnosis as just unknown leukemia or lymphoma. Although the present study was a large cohort, data collection regarding family history of HTLV-1–associated diseases was insufficient because one-half of the participants did not know their family HTLV-1 status. Further detailed data collection is needed to confirm the characteristics of HTLV-1 proviral load levels by family histories among asymptomatic HTLV-1 carriers, as this is necessary to determine genetic determinants of HTLV-1–associated diseases.

HTLV-1 carriers have various comorbidities, such as infectious, autoimmune, and malignant diseases.[4,25,35-38] In the present study, 45 participants had various infectious diseases at enrollment (Table

Table 4. Frequency of subjects who developed ATL by demographic characteristics and by VL level

Demographic characteristics	No. of subjects	No. of ATLs (% of subjects)	Frequency of ATL by VL level, n (% of subjects in each quartile in Table 2)		
			Quartiles 1 and 2 (VL: < 1.60)*	Quartile 3 (VL: 1.60-4.54)	Quartile 4 (VL: ≥ 4.54)
Total	1218	14 (1.1)	0	1 (0.3)	13 (4.3)
Sex					
Male	426	4 (0.9)	0	1 (1.1)	3 (2.0)
Female	792	10 (1.3)	0	0	10 (6.4)
Age, y					
Younger than 40	167	0	—	—	—
40-49	200	2 (1.0)	0	0	2 (3.7)
50-59	273	3 (1.1)	0	0	3 (3.7)
60-69	260	3 (1.2)	0	0	3 (4.5)
70 or older	318	6 (1.9)	0	1 (1.3)	5 (6.5)
First opportunity for HTLV-1 testing					
Screening	661	4 (0.6)	0	0	4 (2.8)
Revelation of HTLV-1–positive family	134	2 (1.5)	0	0	2 (6.9)
During treatment for other diseases	265	7 (2.6)	0	1 (1.8)	6 (7.0)
Unknown	158	1 (0.6)	0	0	1 (2.0)
Family history of HTLV-1–related diseases					
Absence or carrier/HU/HAU only	385	5 (1.3)	0	1 (1.0)	4 (5.0)
HAM/TSP	9	0	—	—	—
ATL	102	3 (2.9)	0	0	3 (9.7)
Leukemia or lymphoma	36	0	0	0	0
Unknown family history	681	6 (0.9)	0	0	6 (3.4)
Comorbidity					
Absence	961	10 (1.0)	0	0	10 (4.1)
Infectious diseases	45	1 (2.2)	0	0	1 (5.9)
Autoimmune diseases	29	0	—	—	—
Malignant diseases	80	1 (1.3)	0	0	1 (5.9)
Skin diseases	15	1 (6.3)	0	1 (33.3)	0
Other disease	87	1 (1.1)	0	0	1 (5.3)

ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HU, HTLV-1 uveitis; HAU, HTLV-1–associated uveitis; HAM, HTLV-1 myelopathy; TSP, tropical spastic paraparesis; and —, not applicable.
*The VL was categorized based on quartile cutoff points (the 25th, 50th, and 75th percentiles of the VL distribution) in 1218 HTLV-1 carriers. The unit of VL was copies/100 PBMCs.

1). We found that the median proviral load of these participants was significantly higher than that of those with no comorbidity (Table 2). The results of the present study support previous reports indicating higher HTLV-1 proviral loads in HTLV-1 carriers with comorbid *Strongyloides stercoralis* or bladder and kidney infections than those without such infections.[25,35,36] HTLV-1 carriers with rheumatoid arthritis or connective tissue disease and those with myelodysplastic syndromes carrying HLA-A26 were also reported to have higher HTLV-1 proviral loads compared with the median proviral load of those without such diseases.[37,38] In the present study, however, the median proviral load was not significantly high in those with autoimmune and malignant diseases. Further studies are required to find other predisposing factors affecting the proviral load level in each person.

A high HTLV-1 proviral load is currently considered as one of the main indicators for the progression to ATL.[20,28] In the present study, 14 participants of asymptomatic HTLV-1 carriers progressed to overt ATL as of 2009, all of whose baseline proviral load levels were high (range, 4.17-28.58 copies/100 PBMCs; Table 3). Therefore, we suggest that those with a high proviral load level (~ > 4 copies/100 PBMCs) are in a high-risk group for developing ATL (this group accounted for ~ 29% of the cohort). Multivariate Cox analyses confirmed that a higher proviral load level was a strong factor in the development of ATL (Table 5). This result strongly supports previous small-scale studies.[20,28] However, the role of the high proviral load level still remains unclear because the majority of asymptomatic carriers with a high HTLV-1 proviral load level in the present study remain carrier status. In the present study, male gender was not a significant risk factor for ATL, even though the median proviral load was significantly higher in males than in females. A high HTLV-1 proviral load is also reported to be associated with HAM/TSP.[20,21,27] These findings suggest that a high

Table 5. Cox proportional hazards modeling of risk factors for ATL development

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P	HR (95% CI)	P
Male sex (vs female)	0.74(0.23-2.37)	.61	0.38(0.12-1.18)	.09
Square-root transformed VL per unit increase	2.55(1.91-3.41)	< .001	3.57(2.25-5.68)	< .001
Age per 5-year increase from 40 y	1.29(0.94-1.59)	.15	1.67(1.12-2.50)	.012
Family history of ATL (vs others)	2.68(0.80-8.98)	.11	12.1 (2.26-64.7)	.004
First opportunity for HTLV-1 testing during treatment of other diseases (vs others)	3.40(1.12-10.25)	.03	4.16(1.37-12.6)	.012

Analyses were performed using robust sandwich variance estimates.
ATL indicates adult T-cell leukemia; HTLV-1, human T-cell leukemia virus type 1; VL, HTLV-1 proviral load; HR, hazard ratio; and CI, confidence interval.

proviral load alone is not a unique predictive marker for ATL. In addition, the present study showed that the median proviral load level at enrollment was lower in those who developed aggressive types of ATL (5.1 copies/100 PBMCs) than that in those who developed smoldering types of ATL (11.4 copies/100 PBMCs; $P = .02$). This also suggests that a high proviral load alone is not a predictive marker for aggressive types of ATL.

In the present study, multivariate Cox analysis indicated that increased age, family history of ATL, and first opportunity to learn of HTLV-1 infection during treatment of other diseases were also independent risk factors for the development of ATL, after adjusting for proviral load (Table 5). This suggests that multiple risk factors (including unknown factors) are related to the progression from HTLV-1 carrier status to ATL. The reason why "opportunity to learn of HTLV-1 infection during treatment of other diseases" was an independent risk factor is unknown. The findings that more advanced states of HTLV-1 carriers (ie, an intermediate state[6] and a preleukemic state[13]) tend to be complicated by various comorbid diseases and that HTLV-1 carriers with various comorbid diseases had higher HTLV-1 proviral loads[25,35-38] could in part explain the reason.

Some prospective studies serially evaluated HTLV-1 proviral loads in HTLV-1 carriers and reported that their proviral load level was relatively stable over time with a certain level of fluctuations for persons.[25,26,28] Taylor et al reported that proviral loads of 20 HTLV-1 carriers were stable over a mean of 27 months, even though 9 carriers with various comorbidities showed high proviral load levels.[25] Meanwhile, an increasing proviral load was observed before progression to HAM/TSP and ATL.[27,28] However, there remain more questions how much of the fluctuations in proviral load over time could predict disease progression over the natural fluctuations within persons. Factors other than the proviral load level might be influencing the development of HTLV-1–associated diseases. Future studies should perform serial evaluations of HTLV-1 proviral loads by considering risk factors that have been confirmed in the present study.

The present study has several limitations. The number of ATL events was very small to obtain a conclusive result. However, we have a confidence for our results because we used a robust variance estimate in the multivariate analysis and because 2 validity analyses confirmed the original results. Data collection was insufficient for some items in the questionnaire. To resolve this issue, we will need to administer the questionnaire repeatedly. Our study design did not include enough information for evaluating the development of HAM/TSP. The follow-up duration is too short with regard to the natural history of ATL that has a long latency. Further follow-up of this cohort and similar prospective investigations should provide data needed to support more detailed conclusions. We did not compare the proviral loads by place of enrollment because we realized that many HTLV-1 carriers have migrated from the southern area to the metropolitan area.[39] The migration of HTLV-1 carriers has raised some public health issues in Japan. Screening for HTLV-1 in pregnant women and prevention programs for mother-to-child transmission of HTLV-1 are conducted in endemic areas[40,41] but not in metropolitan areas, which could introduce a higher chance of new HTLV-1 infections in the metropolitan area. To date, there is no nationwide program for preventing new HTLV-1 infections in Japan. Further nationwide studies are needed to determine the precise numbers of HTLV-1 carriers and to prevent HTLV-1 infection.

In conclusion, the present cohort study of 1218 asymptomatic HTLV-1 carriers provided detailed distributions for HTLV-1 proviral loads regarding the host-specific characteristics and the associations with the development of ATL. We confirmed that a higher proviral load levels (especially $\sim > 4$ copies/100 PBMCs), advanced age, family history of ATL, and having the first opportunity to learn of HTLV-1 infection during treatment of other diseases were independent risk factors for progression from carrier status to ATL. Further large-scale epidemiologic studies are needed to clearly identify the determinants of ATL for early detection and rapid cure for HTLV-1–associated diseases.

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Authorship

Contribution: M.I. managed the study database, analyzed data, and wrote the manuscript; T.W. organized the study and managed processing of the samples and measurement of proviral loads; A.U., A.O., K. Uchimaru, K.-R.K., M.O., H.K., K. Uozumi, M.M., K.T., Y. Saburi, M.Y., J.T., and Y.M. were responsible for participant enrollment and data collection; Y. Sagara managed the biomaterial bank; S.H. organized the study and managed the database; S.K. and K.Y. established the study; and all authors critically reviewed the article and approved the final version.

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A complete list of JSPFAD participants is available online in the supplemental Appendix.

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別紙様式第2-1

No. 18

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
				2010. 10. 9	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	日経メディカルオンライン. Available from: http://medical.nikkeibp.co.jp/leaf/mem/pub/hotnews/int/201010/516836.html	公表国 日本
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			

研究報告の概要

○日本の蚊で伝播する新種ウイルス感染症 チクングニヤ熱が4類感染症に指定へ
2010年10月1日に開催された厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することに合意した。4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニヤ熱を検疫の対象となる感染症(検疫感染症)に追加することも合意された。
チクングニヤ熱は日本国内に広く生息するヒトスジシマカの媒介で伝播しうる。急性期の患者における血中のウイルス量は多く、そのような患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニヤ熱が、国内で広まる可能性は十分高いと危惧している。

報告企業の意見	今後の対応
厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニヤ熱を感染症法における4類感染症に追加することに合意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受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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

79

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NEWS

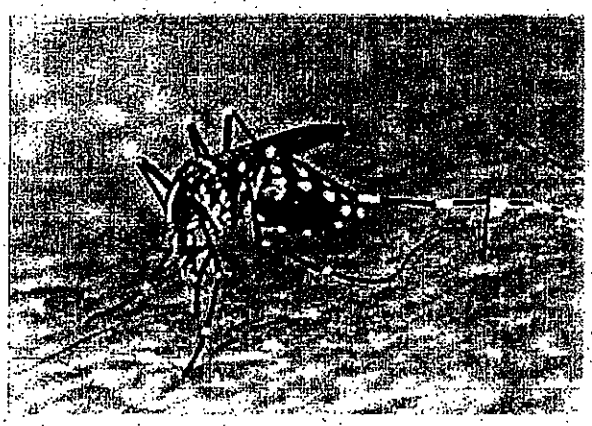
2010. 10. 1

日本の蚊で伝播する新種ウイルス感染症

チクングニア熱が4類感染症に指定へ

小板橋律子＝日経メディカル

10月1日に開催された厚生科学審議会感染症分科会感染症部会は、アジア・アフリカ諸国での流行が問題となっているチクングニア熱を感染症法における4類感染症に追加することを合意した。チクングニア熱は日本に広く生息するヒトスジシマカ（写真）を介して伝播する。



日本に広く分布するヒトスジシマカ。生息の北限は青森八戸付近といわれている（写真提供：国立感染症研究所昆虫医科学部）

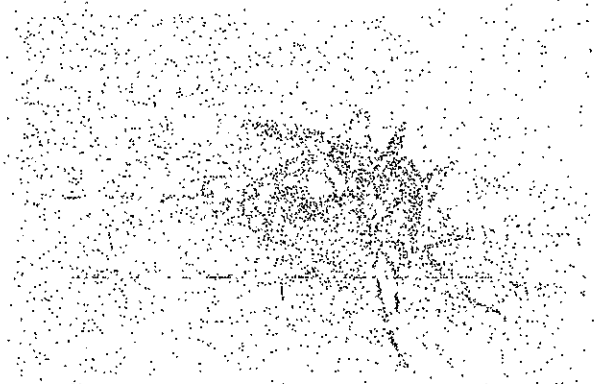
4類感染症に指定されることで、患者を診断した医師には速やかな届け出が義務づけられる。さらに今回の部会では、チクングニア熱を検疫の対象となる感染症（検疫感染症）に追加することも合意された。

チクングニア熱は、チクングニアウイルスにより発症する急性熱性疾患で、発熱、全身倦怠、リンパ節腫脹、頭痛、筋肉痛に加え、手関節などに関節炎を生じる。インドやスリランカなどのアジア諸国で流行しており、日本においても、18人のチクングニア熱が報告されており、その全員が東南アジアなどへの渡航歴がある。

これまでチクングニア熱は死亡例の報告がほとんどなかったが、近年流行している株による感染では、呼吸不全や心不全、髄膜脳炎、劇症肝炎、腎不全などによる死亡例が報告されている。突然変異によって病原性の高いウイルス株に変異している可能性を指摘する専門家もいる。

チクングニア熱は、日本国内に広く生息するヒトスジシマカの媒介で伝播する。急性期の患者における血中のウイルス量は多く、そのような患者を刺した蚊を介してさらに感染者が広まる危険性がある。そのため感染症部会は、日本に持ち込まれたチクングニア熱が、国内で広まる可能性は十分に高いと危惧している。実際、2007年にはイタリアで、インドへの渡航歴がある1人の患者から感染が広まり、約250人を超える感染患者が出ている。今年9月末にはフランスで、海外への渡航歴がないチクングニア熱患者が報告された。

日経BP社



別紙様式第2

別紙(2)-2

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 2010年12月16日	新医薬品等の区分 該当なし	厚生労働省処理欄
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販売名（企業名）	①ベリナート P ②ベリナート P 静注用 500 （CSL ベーリング株式会社）					

研究報告の概要

2009 年のインフルエンザ A ウイルス H1N1 の世界的流行が未だ猛威をふるっている。これまで、輸血による感染症例は報告されていないが、インフルエンザのウイルス血症症例が確認されていることで、血液製剤同様血漿分画製剤の安全性への懸念が高まっている。血漿分画製剤の安全性を評価するため、このウイルスの除去効果について、製造で使用される特定のウイルスクリアランス工程が調査された。
本研究では、インフルエンザ A ウイルス H1N1 株 A/NWS/33(H1N1)がモデルウイルスとして選択された。アルブミンの製造工程において、パスツリゼーションおよびフラクションⅣ分画により H1N1 は不活性化された（対数減少係数 ≧5.34 および ≧2.53）。また、静注用免疫グロブリンの製造工程においては、フラクションⅢ分画によって効果的に除去され（対数減少係数 ≧4.66）、低 pH インキュベーションおよびパスツリゼーションによって不活性化された（対数減少係数 ≧5.41 および ≧4.65）。第Ⅷ因子の製造工程では、0.3%リン酸トリ-n-ブチルと 1.0%トリトン X-100 を用いた S/D 処理で 1 分以内に不活化され（対数減少係数 ≧3.54）、また 98℃乾燥加熱でも 10 分以内に不活化された（対数減少係数 ≧4.36）。アンチトロンビンⅢの製造工程においても、Viresolve NFP フィルターで除去され（対数減少係数 ≧4.92）、パスツリゼーションで不活化された（対数減少係数 ≧3.75）。
この研究を通じて、H1N1 は、一般に用いられるウイルスクリアランス工程（低温エタノール分画、パスツリゼーション、低 pH 処理、S/D 処理、乾燥過熱処理、ウイルスろ過工程）により、効果的に不活化・除去されることがわかった。これは、様々なモデルウイルスや関連ウイルスを用いて種々の血漿分画製剤で試験されたときに得られる通常の結果と一致している。また、限られた種類のモデルウイルスによる特定の工程を検証するために用いられる一般的な方法を支持するものであった。これらの結果より、ウイルスバリデーション試験を含む血漿分画製剤の製造工程は、高い安全性をもたらす十分な H1N1 除去能力を持っていることが示された。

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
今回の研究で、インフルエンザAウイルスH1N1は、通常の血漿分画製剤の製造工程において、不活化・除去されていることがわかった。よって、本剤の製造工程においてもインフルエンザAウイルスH1N1は不活化・除去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。

ABJ20102O3

Biologicals 38 (2010) 652–657

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Inactivation and removal of influenza A virus H1N1 during the manufacture of plasma derivatives

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ABSTRACT

Although transmission of pandemic influenza A virus H1N1 2009 is still occurring globally, little has been reported about how this outbreak has affected the safety of plasma derivatives. To evaluate the safety of plasma derivatives, dedicated virus clearance processes used during their production were investigated for their effectiveness in eliminating this virus of recent concern. In this study, influenza A virus H1N1 strain A/NWS/33 (H1N1) was chosen as a surrogate. H1N1 was completely inactivated by fraction IV fractionation as well as pasteurization during the manufacture of albumin. H1N1 was also effectively removed into the precipitate by fraction III fractionation and completely inactivated by low pH incubation as well as pasteurization during the manufacture of intravenous immunoglobulin. H1N1 was completely inactivated within 1 min of solvent/detergent treatment using 0.3% tri (n-butyl) phosphate and 1.0% Triton X-100 and also completely inactivated within 10 min of dry-heat treatment at 98 °C during the manufacture of factor VIII. H1N1 was completely removed by virus filtration process using Viresolve NFP filter and also completely inactivated by pasteurization during the manufacture of antithrombin III. These results indicate that all the virus clearance processes commonly used have sufficient H1N1 reducing capacity to achieve a high margin of safety.

1. Introduction

The 2009 flu pandemic is a global outbreak of a recently emerged novel influenza virus. The virus was identified to be a swine-origin influenza A virus H1N1 [1–3]. According to World Health Organization monitoring report of July 2, 2010, more than 214 countries have reported laboratory confirmed cases of pandemic influenza A virus H1N1 2009, including over 18,239 deaths [4]. This virus has a hemagglutinin (HA) gene that is derived from the 1918 swine influenza virus and other genes from human, avian, and Eurasian swine influenza viruses.

H1N1, H1N2 and H3N2 subtype swine influenza viruses and highly pathogenic avian influenza virus H5N1 have occasionally infected humans before but such zoonotic transmission events did not lead to sustained human-to-human transmission in the manner that the swine-origin influenza virus has done [5]. Transmission of pandemic influenza A virus H1N1 2009 is still occurring globally. There are concerns that the virus may reassort with existing human influenza virus giving rise to more transmissible or more pathogenic viruses. The virus appears to retain the potential to transmit back to swine and thus continued reassortment with swine viruses is a cause for concern.

Although the occurrence of influenza virus in the blood has been reported in some previous studies [6–9], no cases of transfusion transmission of influenza have been reported to date [10]. However, the confirmed human cases of influenza viremia have raised concerns about the safety of plasma derivatives as well as labile blood products for transfusion [10,11]. The potential for transmission of pandemic influenza H1N1 through blood transfusion remains unknown because there is limited information available on pandemic (H1N1) 2009 virus viremia, especially during the asymptomatic period. At this time, the pandemic (H1N1) 2009

Abbreviations: H1N1, Influenza A virus H1N1 strain A/NWS/33; HA, Hemagglutinin; NA, Neuraminidase; MDCK, Madin–Darby canine kidney; CPE, Cytopathic effect; CPMP, Committee for Proprietary Medicinal Products; IVIG, Intravenous immunoglobulin; S/D, Solvent/detergent; Factor VIII, Antihemophilic factor VIII; TnBP, Tri (n-butyl) phosphate; ATIII, Anti-thrombin III.

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virus has not been isolated from blood or serum of asymptomatic, infected individuals.

The manufacturing processes of plasma derivatives must include virus inactivation and/or removal processes to ensure viral safety, because plasma derivatives are manufactured from pooled human plasma. Specific viral inactivation processes such as solvent/detergent (S/D) treatment, pasteurization, dry-heat treatment, and low pH incubation are the cornerstone in ensuring a sufficient margin of safety of plasma products. Fractionation of plasma by ethanol precipitation and protein purification by chromatography contribute to the removal of viruses by partitioning. Nanofiltration is a specific approach to eliminate viruses [12,13]. Validation of the process for viral inactivation and/or removal plays an essential and important role in establishing the safety of plasma derivatives [14,15].

To date, there is no information about the inactivation and removal of influenza A virus H1N1 during the manufacture of plasma derivatives. To evaluate the safety of plasma derivatives against influenza A virus H1N1, dedicated virus clearance processes used during the production of plasma derivatives were investigated for their effectiveness in inactivating and/or removing this virus of recent concern. In this study, influenza A virus H1N1 strain A/NWS/33 (H1N1) was chosen as a model for pandemic influenza A virus H1N1 2009.

2. Materials and methods

2.1. Preparation and titration of influenza A virus H1N1

In this study, H1N1 strain A/NWS/33 (ATCC VR-219) was chosen as a surrogate of pandemic influenza A virus H1N1 2009 [16]. For the propagation and titration of H1N1, Madin–Darby canine kidney (MDCK) cells (KCLB 10034) were used. MDCK cells were grown in a high glucose Dulbecco's modified Eagle's medium (Hyclone, Thermo Fisher Scientific Inc., Logan, USA) containing 10% fetal bovine serum.

An aliquot from each sample from the virus inactivation studies and an appropriate control were titrated immediately after being collected in 7-fold serial dilutions to the end point using a quantal 50% tissue culture infectious dose ($TCID_{50}$) assay [16,17]. When no infectious virus was detected, the virus titer was calculated using a theoretical minimum detectable level of infectious virus, with a 95% upper confidence level. As a part of the virus validation protocol, cytotoxicity, interference, and load titer tests were performed. The load titer assays were performed to determine precisely the point at which spiking the virus into the starting material resulted in a loss in the virus titer. The virus log reduction factor was defined as the $\log_{10}$ of the ratio of the virus loads in the spiked starting and post process materials, in accordance with Committee for Proprietary Medicinal Products (CPMP) guidance [15]. All the virus inactivation experiments were carried out in duplicate and mean values are given.

2.2. Downscaled manufacturing processes for plasma derivatives

To ensure that the performance of the scale-down process was representative of the production situation, critical process parameters and selected product parameters were compared. To ensure that the performance of the scale-down processes of fraction IV precipitation and fraction III fractionation was representative of the production scale, the physico-chemical properties, such as the recovery yield of protein, pH, temperature, and ethanol concentration, were compared. To ensure that the scale-down pasteurization procedures were equivalent to those used in the manufacture of 20% albumin and immunoglobulin in production facilities, the physico-chemical properties, such as the pH, concentrations of proteins, and aggregate contents, were compared. For the validation of the scale-down process of S/D treatment, the concentration of inactivating agents was evaluated. To ensure that the scale-down dry-heat procedure was equivalent to that used in the manufacture of factor VIII in production facility, the physico-chemical properties, such as factor VIII activity, moisture content, and clottable proteins, were compared. To ensure that the scale-down nanofiltration and pasteurization procedures were equivalent to those used in the manufacture of ATIII in production facility, the physico-chemical properties, such as ATIII activity, pH, and protein content, were compared. Temperature as a critical process parameter for virus inactivation was monitored throughout all the processes investigated. Process intermediates were obtained from the manufacturing scale and used as starting materials.

2.3. Fraction IV fractionation and pasteurization process for albumin

The partitioning profile of the virus after the fraction IV fractionation was assessed in order to evaluate the efficacy of the fraction IV fractionation process in eliminating H1N1. Fraction IV4–2 was prepared by adding 34.7 ml of 95% ethanol to 80 ml of fraction IV4-1, while maintaining the temperature at −5.5 °C, over a 7 h period. The final ethanol concentration reached was 40%. An 80 ml aliquot of the fraction IV4–2 suspension, at −5.5 °C, was then spiked with 8 ml of virus. An 8 ml aliquot of the sample was then immediately removed and titrated. The remaining material was maintained at −5.5 °C over a period of 8 h and then filtered at a pressure of 1.0–1.5 kg/cm^2 using 0.2 μm Supra-80 membranes (Seitz-Schenk, Germany). The resulting filtrate fraction was collected and the retained fraction IV paste was resuspended in cell culture medium with an equivalent volume to that of the filtrate. All samples were neutralized to pH 6.5–7.5 on collection where required and an aliquot from each sample was immediately titrated.

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature, that is just below the temperature specified for manufacturing. A 60 ml aliquot of the final 20% albumin solution containing a stabilizer (13.3 mg sodium caprylacid and 19.7 mg acetyl tryptophane per 1 g albumin) was spiked with 6 ml of virus stock solution and then a 6 ml load sample was removed for titration. The remaining material was heated in a water bath and equilibrated to 58 ± 0.5 °C. Samples were removed at different times over 10 h. An aliquot of each sample was immediately titrated.

2.4. Fraction III fractionation, pasteurization, and low pH incubation for intravenous immunoglobulin (IVIG)

The efficacy and mechanism of fraction III fractionation in the clearance of H1N1 was evaluated. A 96 ml aliquot of the fraction I+II + III-w suspension was spiked with 9 ml of the virus. An 8 ml aliquot of the sample was then immediately removed to determine the viral titer in the spiked starting material. The remaining material was continuously added with 95% ethanol, at a temperature of −20 °C, to a final concentration of 18%, while maintaining the temperature at −5.5 °C, over a 20 h period. The material was then filtered at a pressure of 1.0–1.5 kg/cm^2 using 0.2 μm Supra-80 membranes (Seitz-Schenk; Germany). The resulting filtrate was collected and the retained paste I+III fraction was resuspended in cell culture medium with an equivalent volume to that of the supernatant. An aliquot from each sample was immediately titrated.

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature. The dissolved fraction II solution was dialyzed against water to remove the residual ethanol, and 35 ml of the aliquot solution was then spiked with 3.5 ml of the virus. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. Sorbitol was added to the remaining solution as much as 33% (w/v) and the resulting solution was pasteurized at 58 ± 0.5 °C for 10 h. Samples were removed at different times and then immediately titrated. Protein concentration and pH of IVIG solution during pasteurization were 3.3% and 5.0, respectively.

The efficacy of low pH treatment in inactivating H1N1 was determined. A 54 ml aliquot of IVIG solution was spiked with 6 ml of the virus. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. pH of the remaining solution was adjusted to 3.9 and then the solution was incubated at 25 °C for 14 days. Samples were removed at various times and then immediately titrated. Protein concentration during low pH treatment was 5%.

2.5. S/D and dry-heat treatments for antihemophilic factor VIII (factor VIII)

To validate the effectiveness and robustness of S/D treatment in inactivating H1N1, 5 ml of the virus stock was spiked into 45 ml of pre-S/D solution (supernatants of solubilized cryoprecipitate) equilibrated at 22 °C. A 10 ml aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. The remaining solution was treated with tri (n-butyl) phosphate (TnBP) and Triton X-100, with the final concentration of 0.3% (v/v) and 1.0% (v/v), respectively and then incubated at 22 °C. Samples were taken at indicated time periods, diluted 128-folds with cell culture medium upon being collected to stop the cytotoxic and interfering effects of the S/D, and then titrated immediately. To assess further the robustness of S/D treatment, the virus-spiked solution was treated with the concentration of 50% and 25% of the standard S/D concentrations. Protein concentration and pH of the cryoprecipitate solution during S/D treatment were 13.6 mg/ml and 7.1, respectively.

The effectiveness of dry-heat treatment at 100 °C for 30 min in inactivating H1N1 was evaluated. As the worst-case condition for dry-heat treatment, 98 ± 0.5 °C was adopted. The virus stock was spiked to the final factor VIII complex solution as 10% (v/v) of the total volume of the material. The virus-spiked samples were distributed in final containers at 10 ml/vial. As reference samples for monitoring the temperature, factor VIII activity, and residual moisture content, the factor VIII solution, without the addition of virus, was filled into the vials. After lyophilization, the residual moisture content of the freeze–dried factor VIII was determined by a loss on drying method. Titers of viruses were measured before and after lyophilization. The lyophilized samples were loaded into a convection-drying oven (Korea Science, Seoul, Korea), equilibrated at 30 °C. The dry-heat treatment was then processed for 30 min at 98 ± 1 °C. As soon as the temperature reached 98 °C, the incubation period of dry heating was initiated. Samples were removed at different times, and the titers of the viruses were then measured.

2.6. Pasteurization and nanofiltration for anti-thrombin III (ATIII)

The effectiveness of pasteurization (heat treatment at 60 °C for 10 h) in inactivating H1N1 was determined. Virus inactivation was investigated at 58 °C as the worst-case process temperature. A 5 ml of the virus stock was spiked to 45 ml of purified bulk solution. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. 40 ml of trisodium citrate (1 M, pH 7.0) was added to the remaining solution as the stabilizer and the resulting solution were pasteurized at 58 ± 0.5 °C for 10 h. Samples were removed at different times and then immediately titrated. Protein concentration and pH of ATIII during pasteurization were 4.5 mg/ml and 7.2, respectively.

To evaluate the effectiveness and robustness of the Viresolve NFP (Millipore, Jaffrey, USA) filtration process in eliminating H1N1, a Viresolve NFP disk membrane (active filtration area of 3.5 cm^2) was used to simulate the process performance of the production scale cartridges at 20 °C. ATIII solution was pre-filtered using a 0.1-μm membrane (Millex-VV, Millipore, Cork, Ireland). This pre-filtration was performed within 5 min prior to spiking the test solution with the virus. 47.5 ml of the pre-filtered ATIII solution was spiked with 2.5 ml of the virus stock solution. A 10 ml of the aliquot sample was then immediately removed to determine the viral titer in the spiked starting material. The virus-spiked ATIII solution was then filtered using a 0.22 μm membrane (Millex-GV, Millipore, Cork, Ireland) to remove any viral aggregates, particulates, host cells, or viruses bound to proteins. A 10 ml of the aliquot sample was then immediately taken to determine the viral titer in the pre-filtered ATIII solution. Subsequently, 30 ml of the remaining solution was filtered through a Viresolve NFP disk membrane at a constant pressure of 2.0 bar and then the filtrate was immediately titrated. Thereafter, the post-run integrity of the filter was tested by submerging the assembled membrane holder in water for 1 min at 2.7 bar and checking for any leaks as seen by the formation of bubbles. Sterile pressurized air was used during the filtration and the virus filtration process was conducted in a cold chamber at 4–8 °C. Protein concentration and pH of ATIII during nanofiltration were 10 mg/ml and 7.0, respectively.

3. Results

3.1. Inactivation of H1N1 by fraction IV fractionation and pasteurization during the manufacture of albumin

After the separation of the precipitates, the titers of the virus in the resulting filtrate and the fraction IV paste were analyzed (Table 1). No infectious virus was detected in the filtrate, thereby indicating the complete elimination of the virus during fraction IV fractionation. The log reduction factor achieved was ≥2.53. Furthermore no infectious virus was found to be present in the paste. These results indicate that all the spiked viruses were inactivated during fraction IV fractionation by the addition of ethanol, thus suggesting that the mechanism of reduction was inactivation rather than partitioning

Table 1
Inactivation of H1N1 by fraction IV fractionation and pasteurization during the manufacture of albumin.

Process	Sample	Total virus titer (Log_{10} $TCID_{50}$)
Fraction IV fractionation	Starting material spiked with virus	6.68
	Fraction IV supernatant	≤4.15
	Fraction IV paste	≤2.97
	Log reduction factor	≥2.53
Pasteurization at 58 °C	Starting material spiked with virus	7.47
	0.5 h after pasteurization	3.00
	1 h after pasteurization	≤2.13
	2 h after pasteurization	≤2.13
	5 h after pasteurization	≤2.13
	10 h after pasteurization	≤2.13
	Log reduction factor	≥5.34

under these experimental conditions. To confirm the mechanism of H1N1 elimination during fraction IV fractionation, the inactivation kinetics of H1N1 under the conditions of fraction IV fractionation (ethanol 40%, pH 6.0, −5.5 °C) were studied. H1N1 was inactivated from an initial titer of 7.90 $\log_{10}$ $TCID_{50}$ to undetectable levels within 30 min of treatment (data not shown).

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 1). H1N1 was inactivated from an initial titer of 7.47 $\log_{10}$ $TCID_{50}$ to 3.00 $\log_{10}$ $TCID_{50}$ after 0.5 h of treatment and then completely inactivated to undetectable levels within 1 h of treatment. The log reduction factor obtained was ≥5.34.

3.2. Partitioning and inactivation of H1N1 by fraction III fractionation, pasteurization, and low pH incubation processes during the manufacture of IVIG

H1N1 was effectively removed during the fraction III fractionation process (Table 2). After the separation of the precipitates, no infectious H1N1 was detected in the filtrate but a major quantity of virus was recovered in the waste fraction III paste. The log reduction factor achieved was ≥4.66.

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 2). H1N1 was completely inactivated from an initial titer of 7.21 $\log_{10}$ $TCID_{50}$ to undetectable levels within 0.5 h of incubation. The log reduction factor obtained was ≥4.65.

The efficacy of low pH incubation at pH 3.9 and 25 °C for 14 days in inactivating H1N1 was determined. H1N1 was inactivated instantaneously from an initial titer of 7.54 $\log_{10}$ $TCID_{50}$ to undetectable levels within 1 min of incubation (Table 2). The log reduction factor obtained was ≥5.41.

3.3. Inactivation of H1N1 by S/D treatment and dry-heat treatment during the manufacture of factor VIII

H1N1 was inactivated to undetectable levels within 1 min of S/D treatment (Table 3). The log reduction factor achieved was ≥3.54. To further assess the robustness of S/D treatment, the kinetics of inactivation of H1N1 was studied at decreased concentrations of TnBP and Triton X-100. Virus solutions were treated with the concentration of 50% and 25% of the standard manufacturing conditions and then the results were compared with that obtained with the standard S/D concentration (data not shown). S/D treatment with the reduced concentrations was also very effective in inactivating H1N1. H1N1 inactivation was complete within 1 min even when the concentrations of TnBP and Triton X-100 were reduced to 25% of the standard concentrations.

The effect of dry-heat treatment at 98 °C after lyophilization on the inactivation of H1N1 was measured (Table 3). H1N1 was highly resistant to the lyophilization process. The titer of H1N1 was slightly reduced from 6.96 $\log_{10}$ $TCID_{50}$ to 6.35 $\log_{10}$ $TCID_{50}$ during the lyophilization, with an average log reduction factor of 0.61. The remaining H1N1 after lyophilization was completely inactivated to undetectable levels within 10 min of dry-heat treatment. The log reduction factor obtained was ≥4.36. The mean residual moisture values of factor VIII were 0.70% after lyophilization and 0.69% after dry-heat treatment.

Table 2
Removal and inactivation of H1N1 by fraction III fractionation, pasteurization, and low pH incubation during the manufacture of IVIG.

Process	Sample	Total virus titer (Log_{10} $TCID_{50}$)
Fraction III fractionation	Starting material spiked with virus	8.82
	Fraction III supernatant	≤4.16
	Fraction III paste	7.51
	Log reduction factor	≥4.66
Pasteurization at 58 °C	Starting material spiked with virus	7.21
	0.5 h after pasteurization	≤2.56
	1 h after pasteurization	≤2.56
	2 h after pasteurization	≤2.56
	5 h after pasteurization	≤2.56
	10 h after pasteurization	≤2.56
	Log reduction factor	≥4.65
Low pH treatment at pH 3.9 and 25 °C	Starting material spiked with virus	7.54
	1 min after treatment	≤2.13
	1 day after treatment	≤2.13
	5 day after treatment	≤2.13
	9 day after treatment	≤2.13
	14 day after treatment	≤2.13
	Log reduction factor	≥5.41

Table 3
Inactivation of H1N1 by S/D treatment and dry-heat treatment processes during the manufacture of factor VIII.

Process	Sample	Total virus titer (Log_{10} $TCID_{50}$)
0.3% TNBP + 1% Triton X-100 treatment	Starting material spiked with virus	7.50
	1 min after S/D treatment	≤3.96
	5 min after S/D treatment	≤3.96
	10 min after S/D treatment	≤3.96
	30 min after S/D treatment	≤3.96
	Log reduction factor	≥3.54
Dry-heat treatment at 98 °C	Starting material spiked with virus	6.96
	After lyophilization	6.35
	10 min after dry-heat treatment	≤2.60
	20 min after dry-heat treatment	≤2.60
	30 min after dry-heat treatment	≤2.60
	Log reduction factor	≥4.36

3.4. Removal and inactivation of H1N1 by nanofiltration and pasteurization during the manufacture of ATIII

To evaluate the effectiveness and robustness of the Viresolve NFP filtration process in eliminating H1N1, three different lots of Viresolve NFP disk membranes were challenged with H1N1 (Table 4). None of the infectious virus was detected in the filtrate of any of the three filter lots tested, indicating that H1N1 was completely removed to undetectable levels. The log reduction factor achieved was ≥4.92.

The effectiveness of heat treatment at 58 °C as the worst-case process temperature for pasteurization in inactivating H1N1 was determined (Table 4). H1N1 was completely inactivated from an initial titer of 6.65 $\log_{10}$ $TCID_{50}$ to undetectable levels within 0.5 h of incubation. The log reduction factor obtained was ≥3.75.

4. Discussion

Ethanol is known to be both bactericidal and virucidal and is the principal precipitation agent in cold ethanol fractionation used in the manufacture of plasma products. The highest concentration of ethanol in the fractionation is 40% and in some steps only 8–25%. In addition to the virucidal effect of ethanol, the partitioning of viruses

Table 4
Removal and inactivation of H1N1 by Viresolve NFP filtration and pasteurization during the manufacture of ATIII.

Process	Sample	Total virus titer (Log_{10} $TCID_{50}$)
Viresolve NFP filtration	Starting material spiked with virus	7.35
	Pre-filtered solution	7.20
	Filtrate after Viresolve NFP filtration	≤2.43
	Log reduction factor	≥4.92
Pasteurization at 58 °C	Starting material spiked with virus	6.65
	0.5 h after pasteurization	≤2.90
	1 h after pasteurization	≤2.90
	2 h after pasteurization	≤2.90
	5 h after pasteurization	≤2.90
	10 h after pasteurization	≤2.90
	Log reduction factor	≥3.75

during fractionation is also important in determining the effectiveness of this process in the production of safe plasma products [18]. The mechanism of H1N1 clearance during fraction IV fractionation, which involved the use of a higher ethanol concentration of 40%, was found to be inactivation rather than partitioning. Previous reports have also shown that the mechanism of reduction for the enveloped viruses such as human immunodeficiency viruses (HIV), bovine viral diarrhea virus (BVDV), and bovine herpes virus (BHV) during fraction IV fractionation was inactivation rather than partitioning, however, it was partitioning in the case of the resistant non-enveloped viruses such as hepatitis A virus (HAV), murine encephalomyocarditis virus (EMCV), and porcine parvovirus (PPV) [19–21]. H1N1 was also completely removed during fraction III fractionation. However the major mechanism of H1N1-reduction during fraction III fractionation was found to be partitioning. Partial inactivation of H1N1 was also observed during fraction III fractionation. Although no infectious viruses were detected in the filtrate, the recovery of H1N1 in the fraction III paste was lower than the amount of spiked virus. The difference of total virus titer before and after the fraction III fractionation was about 1.31, which showed that infectivity of H1N1 was also reduced, to a limited extent, by the added ethanol during the fraction III fractionation. This result is consistent with the previous report that the mechanism of reduction in eliminating enveloped viruses such as BHV and BVDV was principle removal but with also some inactivation [22].

A commonly used method for the inactivation of virus contaminants in plasma products is heating in a liquid for at least 10 h at 60 °C [19–21,23]. The inactivation kinetics of H1N1 showed that H1N1 was completely inactivated during the pasteurization process, with no residual infectivity being detected on completion of the pasteurization process resulting in a high level of virus inactivation. The times needed for achieving a complete inactivation of H1N1 in these experimental conditions were 1 h for albumin, 0.5 h for IVIG, and 0.5 h for ATIII. According to these results, it can be concluded that pasteurization process is a robust and effective step in eliminating H1N1. It has been also reported that H1N1 was inactivated to undetectable levels within 5 min, 2.5 min, and 1 min of heat treatment at 70, 80, and 90 °C, respectively [16]. Avian influenza A virus H5N1 was also very sensitive to pasteurization, being completely inactivated within 30 min of pasteurization during the manufacture of albumin [24].

Low pH incubation of IVIG has been reported by a number of investigators to effectively inactivate enveloped viruses such as HIV, BVDV, BHV, herpes simplex virus, cytomegalovirus, vesicular stomatitis virus, and Semliki Forest virus [22,25,26]. H1N1 was extremely sensitive to low pH treatment. H1N1 was inactivated in 1 min of treatment at pH 3.9. Previous report has also shown that avian influenza A virus H5N1 was inactivated instantaneously by the treatment at pH 4.4 or 4.9 [24]. This extreme sensitivity of influenza viruses to low pH was probably due to the low pH-triggered conformational change in influenza virus HA [27]. 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 [24].

One of the most widespread methods for inactivating enveloped virus contaminants in plasma products is the S/D treatment [28,29]. The solvent frequently used in the S/D method is TnBP and the detergent is either Tween 80, Triton X-100, sodium cholate, Tween-20, or Triton X-45. Organic S/D mixtures disrupt the membranes of viruses which have lipid envelopes. The results of inactivation kinetics obtained from the present study showed that S/D treatment is a robust and effective step in eliminating H1N1. This virus was completely inactivated to undetectable levels within 1 min of the 60 min process time by treatment with 0.3% TNBP and 1% Triton X-100. In addition, H1N1 was completely inactivated within 1 min when a concentration of 25% of the standard manufacturing concentration was used. These conditions were chosen as the worst-case that could be encountered in the manufacturing process.

Dry-heat treatment is another of the choices generally recommended for virus inactivation [30–32]. It is known that virus infectivity substantially diminishes after being subjected to lyophilization as well as dry-heat treatment. Therefore, the effects of both of these processes on the inactivation of H1N1 were measured. H1N1 was highly resistant to the lyophilization process, with an average log reduction factor of 0.61. However H1N1 remaining after lyophilization was completely inactivated to undetectable levels within 10 min of dry-heat treatment at 98 °C.

Another potential way to increase the safety of therapeutic biological products is the use of virus-retentive filters [33,34]. H1N1 is an 80–120 nm size pleiomorphic virus [35]. In this experiment, H1N1 was completely removed to levels below the detection limit during the Viresolve NFP (pore-size 20 nm/molecular weight cut off 160 kDa) filtration process for ATIII. This result indicates that the filtration process was a robust and effective method for removal of H1N1 from plasma derivatives. H1N1 would, based on its size, also be expected to be removed by the now widely used virus filters with nominal pore sizes in the 15- to 75-nm range.

Through this study it was found that H1N1 is effectively inactivated or removed by all the commonly used virus clearance procedures, such as cold ethanol fractionation, pasteurization, low pH treatment, S/D treatment, dry-heat treatment, and virus filtration process. This is in agreement with the general results obtained using a range of model and relevant viruses when tested on various plasma products. Also this lends further support to the general strategy used for validating specific processes with a small range of model viruses. These results indicate that the production processes for plasma derivatives, containing validated virus clearance procedures, have a high H1N1-reduction capacity to achieve a high margin of safety.

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No. 14

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
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一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		Wu JY, Lun ZR, James AA, Chen XG., Am J Trop Med Hyg. 2010 Sep;83(3):664-71.	中国

研究報告の概要

○中国のデング熱
デング熱は蚊が媒介する急性感染症であり、深刻な世界的公衆衛生問題となっている。
中国では大規模アウトブレイクが1978年に既に報告されている。1978～2008年の30年間に合計655,324症例が報告され、610名が死亡した。
1990年代以降、デング熱の流行は、南部沿岸地域から北部や西部にまで広がりつつある。
中国本土において、デング熱ウイルスの主要な伝播媒介動物であるヤブカ属の生物学的行動および媒介能が大きく変化してきており、これはおそらく都市化の加速や地球温暖化によるものである。また、人口増加や頻繁な国際旅行もデング熱流行の増加要因となる。デング熱制御への方法は、媒介蚊のコントロール、流行を予測する迅速ウイルス発見システムの設立、地域に密着した教育、そして安全かつ有効なワクチンを開発することである。

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

急速な都市化や地球温暖化の影響により、中国におけるデング熱の流行は南部沿岸地域から北部や西部に広がりつつあり、デング熱制御への対策が必要であるとの報告である。

今後の対応

日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を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。

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Review: Dengue Fever in Mainland China

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Abstract. Dengue is an acute emerging infectious disease transmitted by *Aedes* mosquitoes and has become a serious global public health problem. In mainland China, a number of large dengue outbreaks with serious consequences have been reported as early as 1978. In the three decades from 1978 to 2008, a total of 655,324 cases were reported, resulting in 610 deaths. Since the 1990s, dengue epidemics have spread gradually from Guangdong, Hainan, and Guangxi provinces in the southern coastal regions to the relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces. As the major transmission vectors of dengue viruses, the biological behavior and vectorial capacity of *Aedes* mosquitoes have undergone significant changes in the last two decades in mainland China, most likely the result of urbanization and global climate changes. In this review, we summarize the geographic and temporal distributions, the serotype and genotype distributions of dengue viruses in mainland China, and analyze the current status of surveillance and control of vectors for dengue transmission.

INTRODUCTION

Dengue fever (DF) is an acute infectious disease caused by dengue viruses and transmitted by *Aedes* mosquitoes. This disease is endemic or epidemic in more than 100 countries and regions in Asia, Oceania, America, and Africa, and it is especially prevalent in Southeast Asia, the west Pacific Ocean regions, and southern Africa. The rapid increase of DF incidence in recent years has become a serious public health threat to nearly half of the world's population.[1] The World Health Organization (WHO) estimates that approximately 2.5 billion people worldwide are at risk and 50 million people are infected by dengue viruses each year. Half a million people suffer from dengue hemorrhagic fever (DHF), which results in more than 25,000 deaths (http://www.who.int/tdr/publications/publications/dengue). Dengue is the second-most serious vector-borne disease in the world, only behind malaria in terms of morbidity and mortality.

The first outbreak of DF in mainland China occurred in Guangdong province in 1978. Since then, dengue outbreaks were recorded sequentially in Hainan, Guangxi, Fujian, and Zhejiang provinces.[2] These epidemics have had significant negative impact on the affected population, the society in general, and the development of the economy. The rapid urbanization in China changed the characteristics of DF epidemics. Dengue epidemics have spread from Guangdong, Hainan, and Guangxi provinces in the southern coastal regions to the relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces, with shorter epidemic intervals as compared with those experienced before the 1990s.

PREVALENCE

Geographic distribution. Dengue fever in mainland China is still characterized as an imported epidemic disease, and so far has not been confirmed to be an endemic.[3] Sporadic cases and outbreaks of DF in the southeast coast region, the middle and lower reaches of the Yangzi River were documented in the early 1940s,[4,5] but since then, no cases were reported. In May, 1978, a sudden outbreak of DF occurred in Foshan, Guangdong province, and it was spread to seven adjacent counties and cities where a total of 22,122 cases, including 16 fatalities were reported.[6] In the past 30 years (1978 to 2008), DF outbreaks in varying scales have occurred in China, and a total of 655,324 cases were documented, resulting in 610 deaths. Figure 1 presents the annual cases of DF in the mainland from 1978 to 2008. In Hainan province, the two most severe outbreaks of DF and DHF occurred in 1980 and 1986, resulting in > 600,000 cases with 475 deaths overall. The 1980 outbreak alone caused 454,205 cases.[2] However, no additional DF outbreaks have been reported from Hainan province since 1991.[7] In recent years, Guangdong province has the highest incidence of DF epidemics (Figure 2), with cases reported every year since 1997.[8,9]

Before the 1990s, large-scale epidemics of DF were characterized by a sudden outbreak, fast progression, quick transmission, and were under control within 2 or 3 years of onset (Figure 1). After the major outbreaks, the following DF epidemics would usually become weak either in the incidence or in transmission speed, but the affected area was more extensive. The DF epidemic now spreads gradually from Guangdong, Hainan, and Guangxi provinces in the Southern coastal regions to the relatively Northern regions, including Fujian, Zhejiang, and Yunnan provinces (Figure 3). In Fujian province in particular, a major outbreak with 1,549 cases was reported in 1999 in Fuzhou.[14] In 2004, a DF outbreak with 83 cases reported in Zhejiang province.[11] An outbreak with 56 cases was reported in 2008 in Yunnan province (State Ministry of Public Heath in October 2008, http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm), which was the first outbreak of the disease in this region since 1949.

Currently, no evidence is available to support the presence of epidemic foci in China, and most of the researchers attributed the prevalence of DF to the imported cases.[15–17] However, with the rapid growth of the Chinese economy, international exchanges are more frequent than ever. In the meantime, more than 10% of China's population has moved away from their original residences, mainly from poor rural areas to

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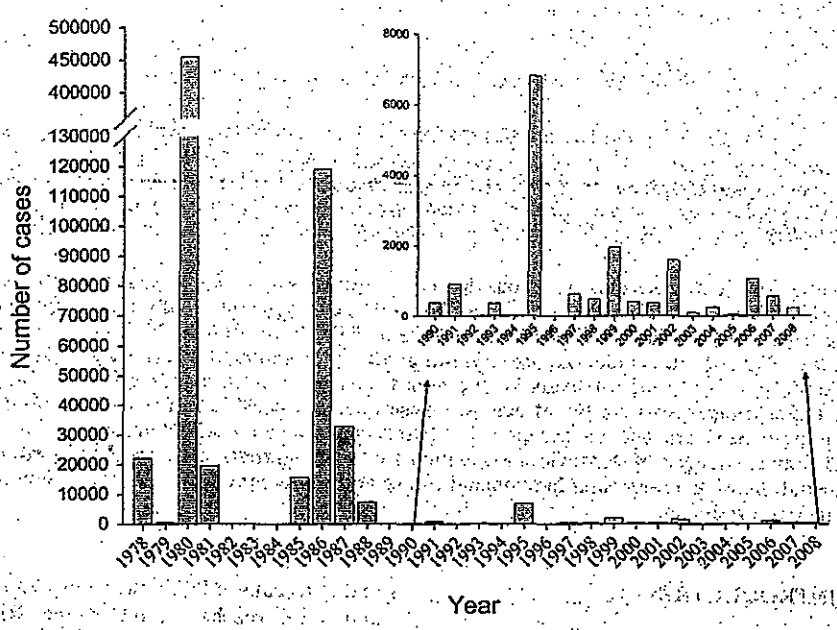

FIGURE 1. Annual number of dengue fever cases in mainland China from 1978 to 2008. Data for 1978–2002 are summarized in Refs. 7–10. Data for 2003–2008 are derived from reports of the State Ministry of Public Heath (http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm).

urban centers, searching for better working and living opportunities. This migration promotes the transmission of infectious diseases and creates major challenges to prevent and control them.[18] It is possible that DF is transforming from an imported to an endemic infectious disease, especially in Guangdong province where the climate favors the survival and transmission of Dengue virus. Although DF in mainland China has not resulted in large epidemics since 1990 (> 10,000 cases), frequent small outbreaks of DF (1,000–7,000 cases) ensure long-term viral circulation in local regions, which has the potential of making DF endemic if no effective intervention is implemented.

Temporal distribution. In mainland China, DF is prevalent mostly in the tropical and subtropical regions (south of 29° north latitude), where the mosquito vectors of dengue viruses breed throughout the year. Figure 4 shows the total number of DF cases reported monthly from 2002 to 2008 (http://www.moh.gov.cn/publicfiles//business/htmlfiles/zwgkzt/pyq/index.htm). January to May is identified as the period of sporadic occurrence, while June to December is recognized as the prevalent period of DF. In general, July is the early stage of the epidemic, which then increases from August to October with 14,487 cases, accounting for 92.34% of the total cases reported within these years. It is documented that the prevalence of virus is associated highly with the breeding activity of the *Aedes* mosquitoes. *Aedes albopictus* is the predominant species in South China. It can breed in various small containers or plants that hold accumulated water (such as tree holes, bamboo

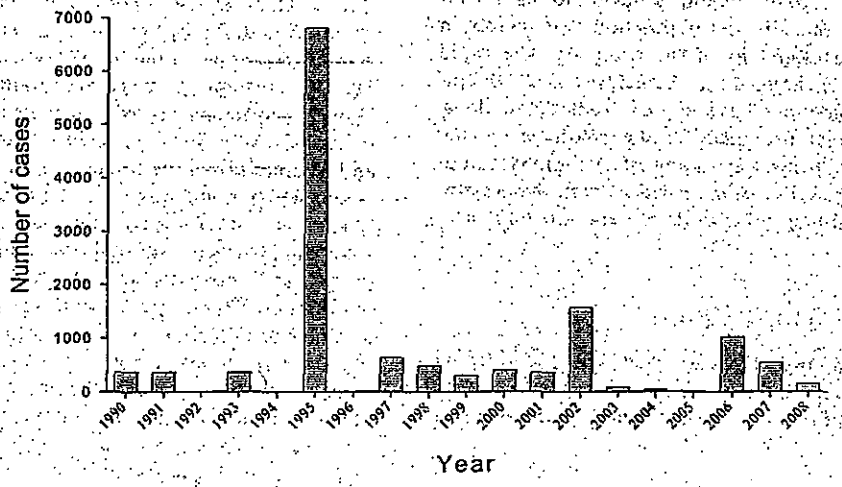

FIGURE 2. Annual number of dengue fever cases in Guangdong Province from 1990 to 2008. The 1990–2000 and 2001–2006 data are from Refs. 8 and 9. The 2007–2008 data are from reports of the State Ministry of Public Health (http://www.moh.gov.cn/publicfiles /business/htmlfiles/zwgkzt/pyq/index.htm).

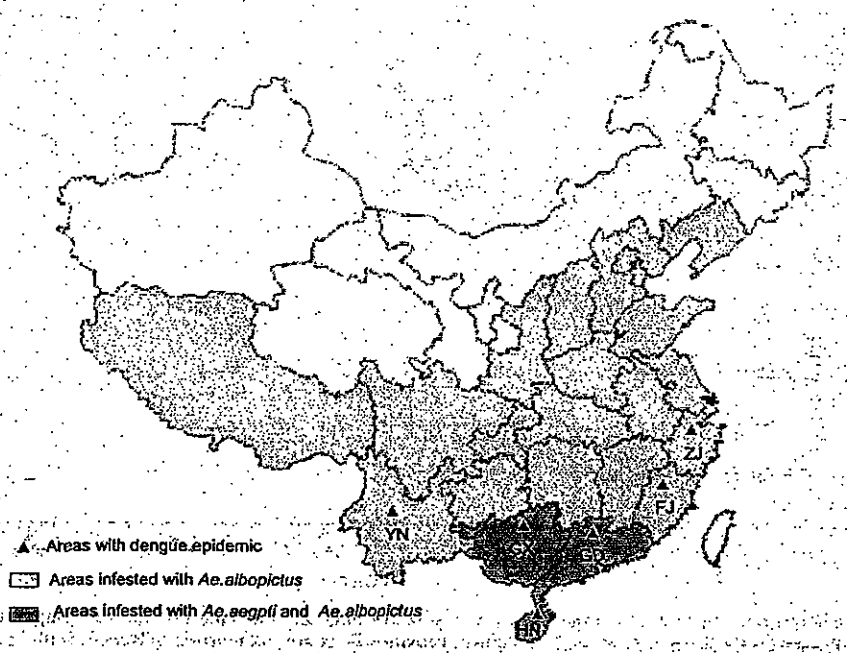

FIGURE 3. Approximate distribution of dengue and *Aedes* mosquitoes in mainland China. ZJ: Zhejiang Province; FJ: Fujian Province; GD: Guangdong Province; GX: Guangxi Province; HN: Hainan Province, YN: Yunnan Province. Data are summarized in Refs. 7, 8, 10–13.

stems, or leaf axils) that are found in gardens or backyards. The large amount of rainfall from July to September increases the breeding places of the mosquitoes. The emergence of large numbers of larvae causes drastic expansion of the mosquito populations and greatly increases the probability of DF epidemic.[19] Yi and others[20] analyzed DF cases in Guangdong province from 1990 to 2001 and *Aedes* surveillance and climate from 1995 to 2001. They showed a clear seasonal pattern of DF occurrence, with a high frequency occurring mostly in hot and humid seasons. Geographic distribution of dengue epidemic has spread gradually from southern (Guangdong, Guangxi, Hainan) to relatively northern and western regions including Fujian, Zhejiang, and Yunnan provinces (Figure 3), which may be associated with the intensifying global warming.[19,21,22] However, the impact of global warming on the spread of vector-borne diseases in tropical and sub-tropical regions in Asia is a subject of debate.[23] Countries or regions experiencing increasing DF should establish as part of their disease-control efforts surveillance that allows an evaluation of the variation in DF incidence and prevalence as the climate in the regions change.[24]

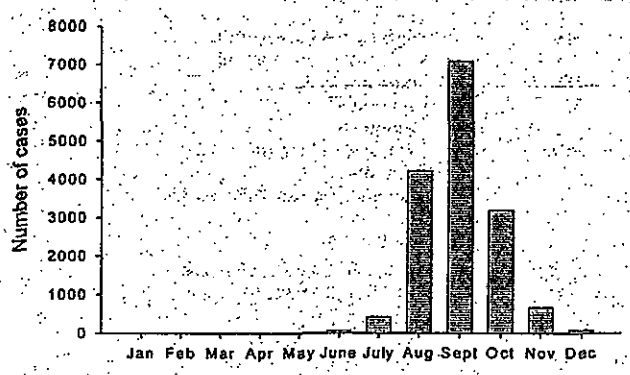

FIGURE 4. Total number of dengue fever cases reported monthly from 2002 to 2008. Data are summarized from (http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm).

Serotype and genotype distribution of dengue viruses. Four serotypes (DV1, DV2, DV3, and DV4) of dengue viruses have been identified and they all are capable of causing DHF and dengue shock syndrome.[12,25] Although the association between the serotype and the severity of the disease is not clear, the DV2 serotype, particularly in the event of superinfection following infection of another serotype, has been shown to correlate with dengue shock syndrome.[25] All four serotypes have caused epidemics in the mainland. Figure 5 shows the geographic distribution of dengue epidemics reflecting a gradual spread from southern to northern regions. Interestingly, no DF epidemics outbreaks have been reported in Guangxi and Hainan province since 1986 and 1991, respectively. The outbreaks in Guangdong province, where the latitude and weather conditions are similar to Guangxi and Hainan, have been reported more frequently since 1990. In the mainland, DV3 was the principal serotype of the viruses reported early (1978), and then DV1 and DV4 were recorded. However, DV1 has become the main serotype since the 1990s. The outbreak of DV4 DF was first reported in Foshan, Guangdong province in 1978[12] and it reappeared here in 1990.[8] The epidemic of DV3 DF was first reported in north Shanbei County of Hainan province in October, 1979; the epidemic then spread north along the west coast to compromise Zhanjiang, Foshan, Guangzhou, Shantou, and Shaoguan of Guangdong province, and then to Beihai and Hepu of Guangxi province, lasting for 3 years.[12] The epidemic caused by DV2 was reported in north Shanbei County, Hainan province in September, 1985, and by the following year it had compromised the entire Island (e.g., Hainan province) and spread further to Guangzhou of Guangdong province and Beihai of Guangxi province, and persisted until 1988.[7] The DV2 caused DF epidemics in

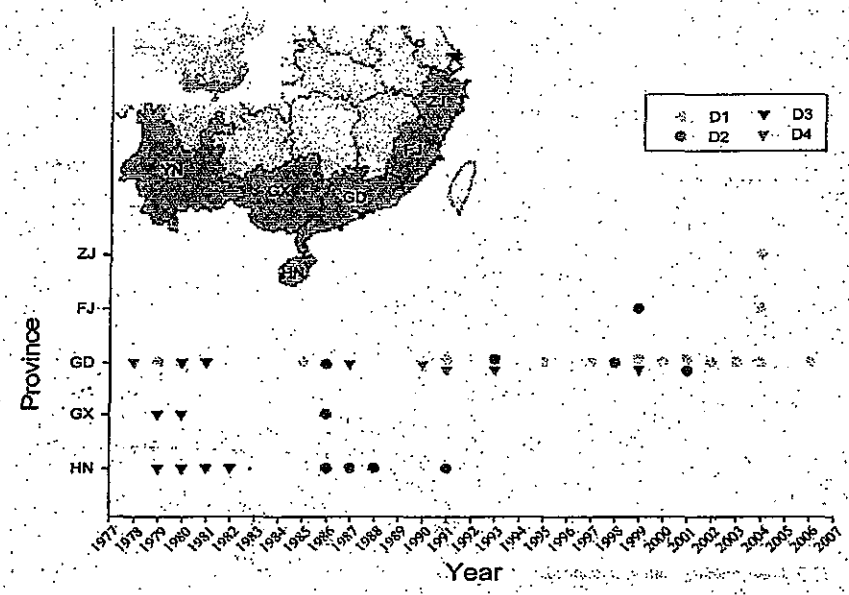

FIGURE 5. The epidemics of four serotypes of dengue virus in mainland China. ZJ: Zhejiang Province; FJ: Fujian Province; GD: Guangdong Province; GX: Guangxi Province; HN: Hainan Province, YN: Yunnan Province. Data are summarized in Refs. 7, 8, 10–13. There were 56 cases of DF in Yunnan Province (2008) according to the report from the Department of Health [http://www.moh.gov.cn/publicfiles/business/htmlfiles/zwgkzt/pyq/index.htm], but no publication regarding the serotype of this virus caused this outbreak was found. This figure appears in color at www.ajtmh.org.

Guangdong province in 1993, 1998, 2001, and in the Fujian province in 1999.[10] In the 1993 epidemic, several cases were found to consist of both DV2 and DV4, which represented the rare cases of concurrent infections in mainland China.[6] The DV1 was responsible for the outbreaks of DF in Guangdong province in 1979 and 1985, respectively,[12] and then several outbreaks caused by the same virus were reported in 1991, and from 1995 to 2006.[8,9]

According to the full-length sequence of the E/NS1 gene, the four serotypes of the virus can be divided into different genotypes. The DV1 virus has five genotypes,[26] DV2 has six,[27] DV3 has four,[28] and DV4 has two genotypes, I and II.[29] Genotyping of the virus strains isolated from different outbreaks has provided some insight into the variation and transmission of the prevalent strains.[26] Analysis of the genotypes of the virus strains in mainland China show complex origins consistent with the interpretation that the viruses might be imported from more than one country (Table 1). A molecular epidemiological analysis comparing the viral gene sequences of the DV1 strains isolated from four DF outbreaks (1991, 1995, 1997, and 1999) in Guangdong province revealed extensive nucleotide variation over the whole genome. The strain GD03/91, isolated in 1991, shows 97% nucleotide identity with the strains GD23/95 isolated in 1995, 93% identity with GD14/97 isolated in 1997, and only 93% identity was identified between strains GD23/95 and GD14/97, indicating that GD03/91 has a closer genetic

TABLE 1
Genotype of dengue virus recognized in mainland China

Serotype	Year	Region	Genotype	Origin (homology)	References
D1	1985	Guangzhou, Guangdong Province	V	Thailand	32
	1991	Guangzhou, Guangdong Province	IV	Southeast Asia, Australia	15,32
	1995	Guangzhou, Guangdong Province	IV	Southeast Asia, Australia	32
		Chaozhou, Guangdong Province	IV	Southeast Asia	15,30
	1997	Chaozhou, Guangdong Province	I	Cambodia	15,30
	1999	Zhongshan, Guangdong Province	I	Cambodia	30
	2002	Guangzhou, Guangdong Province	IV	Australia TI4 strain	33
	2003	Guangzhou, Guangdong Province	I	Cambodia	34
	2006	Guangzhou, Guangdong Province	V	Thailand, Taiwan ThD1 strain	35
	2004	Fujian Province	I	Thailand	36
	2004	Zhengjiang Province	I	Thailand	12
D2	1989	Hainan Province	III	Jamaican or Brazil 90 strain	37
	1998	Nanhai, Guangdong Province	III	Thailand ThNH81/93 strain	15,38
	1993	Fanshan, Guangdong Province	I	Australia TSV01 strain	38,39
	1999	Fujian Province	IV	Indonesia, Sri Lanka	40
	2001	Jiangmen, Guangdong Province	I	Australia TSV01 strain	38
D4	1978	Fanshan, Guangdong Province	II	Indonesia	41
	1990	Guangdong Province	I	Philippines	31

relationship to GD23/95 than GD14/97. The phylogenetic tree is consistent with the hypothesis that strains GD03/91 and GD23/95 derive from Southeast Asia or the Pacific islands.[15] Strains GD14/97 and GD05/99 isolated in 1999 had sequences similar to the Cambodia strain, showing nucleotide and amino acid identities of 98% and 99%, respectively. Phylogenetic tree analysis indicated that the strain GD14/97, GD05/99, and GD23/95 belong to two different genotypes.[30] Fang and others[38] compared the nucleotide sequences of a number of genes between the DV2 strains GD06/93 and GD01/98 isolated from the outbreak in 1993 and 1998, respectively, in Guangdong province and found that these two strains and the strain (04) isolated in Hainan province in 1985 belong to different genotypes. However, GD01/98 shows high similarity to the strain ThNH2P28/93 from Thailand with nucleotide and amino acid sequences at 98% and 100% identity, respectively. Comparison of the sequences among the strain GD01/98, GD06/93, and HN04 was consistent with the interpretation that these three strains originate from different regions. Yao and others[31] analyzed the NS2 gene in DV4 virus isolated in 1978 and 1990 in mainland China, and they found a 96% identity between the 1990 strain and the Philippine strain, and a 96% identity between the strain 7856B2 (isolated in 1978 in mainland China) and the Caribbean strain 814669. Therefore, they presumed that these two strains isolated in mainland China might come from these two regions. These results are consistent with the conclusion that DF epidemics in Guangdong province may be primarily the result of the virus imported from multiple regions. Molecular epidemiological analysis of DF in the last three decades did not identify new variants of dengue viruses in mainland China (Table 1). Therefore, we propose that the rapid expansion of the urban population and the great migration of citizens as well as frequent international travel may result in an increase in the frequency of DF epidemics.

VECTORS

Behaviors. *Aedes aegypti* and *Ae. albopictus* are the two most important mosquito species for transmission of dengue viruses in mainland China. *Aedes aegypti*, a domestic mosquito, can breed inside and outside houses, especially in water-containing vessels in the kitchen or in jars with accumulated rain water. This species is found mostly in the regions south of the 22° north latitude, including the coastal areas in West Guangdong province and the Beibu Gulf of Guangxi Province (Figure 3). *Aedes albopictus* breeds mostly in the wild and depends on accumulated water in various utensils or plants. It is found in nearly one-third of China ranging from Shenyang in the North, Longxian County and Baoji in the Northwest, South Tibet in the Southwest to the South Changjiang regions, where this mosquito is most common (Figure 3).[42] However, the environment and ecology of these mosquitoes has changed greatly because of the rapid urbanization, which may cause significant alteration in the number and types of vector breeding sites. Su and others[43] showed following close monitoring that the Breteau index of *Ae. aegypti* in Haiko city, Hainan province, dropped from 32.81 to 1.73, and the number of *Ae. aegypti*-positive communities reduced from 23 to 2, from 1987 to 2002. Conversely, the Breteau index of *Ae. albopictus* increased gradually from 13.15 to 21.84, and the number of positive communities increased from 19 in 1987 to 23 in 2002. *Aedes albopictus* is considered to be only semi-domestic, but according to a survey by Lin and others,[44] the density of this mosquito inside houses was higher than outside in all of the four monitoring sites. They proposed that the indoor environment provides a suitable breeding place for this mosquito and it has adapted to being a domestic mosquito like *Ae. aegypti*.

Efficiency for virus transmission. The transmission cycles of arboviruses result from horizontal (by adult mosquitoes) and vertical (transovarial) transmission of the mosquito vectors with the significance of each of these components varying for the specific virus. Furthermore, the transmission dynamics of the viruses depend largely on the efficiency of the vector in terms of the susceptibility of mosquitoes to the virus, its subsequent transmissibility, and the transovarial transmission ability of the virus. All of these factors may be affecting *Aedes* transmission of dengue viruses. It is clear that the susceptibility and transmission efficiency of mosquitoes to dengue viruses is affected by the species and strains of mosquito[44–46] and the virus serotypes,[47,48] in addition to the other factors, such as temperature of the environment and the nutrition status of the vector.[49,50]

Temperature may cause alterations in the length of the extrinsic incubation period by affecting viral replication *in vivo*. Within the optimal range (26–35°C), relatively higher temperature (35°C) can promote viral replication *in vivo*, shorten the extrinsic incubation period, and enhance the transmissibility of the virus. Exposure to a relatively lower temperature (≤10°C) alone may reduce the physiological activities of the mosquito, decrease the viral replication rate, thus prolong the extrinsic incubation period and attenuate the transmissibility of the virus.[51] The question then arises over the preservation of the virus in winter and dry seasons when the mosquito hosts are present in low abundance. A possible answer is that the virus can survive in a vector in functional diapause, or has been transmitted to the next generation through the eggs. Lin and others[52] orally infected *Ae. albopictus* with DV1 and DV2 and homogenates prepared from parental and F1–F3 generations. Dengue viruses in these samples were identified following inoculation of C6/36 cell cultures and specific viral antigen was detected by indirect immunofluoresence assay (IFA). These results provide compelling evidence that *Ae. albopictus* could transmit virus to the F2 generation by the eggs. Zhao and others[53] used the same method to infect three geographic strains of *Ae. albopictus* with dengue viruses and the viruses in following generations were detected by IFA and reverse transcription-polymerase chain reaction (RT-PCR). Their result also confirmed that four serotypes of dengue virus are capable of vertical transmission by *Ae. albopictus*. However, the virus can be seldom detected in the vector mosquitoes collected from the field. Fang and others[54] performed RT-PCR of over 6,000 *Ae. albopictus* specimens collected between 1996 and 1998 from three monitoring sites in the suburbs of Guangzhou, Guangdong province, and found none positive for dengue viruses. Similarly, Duan and others[55] collected >300 *Ae. albopictus* from three monitoring sites in Jieyang, Foshan, and Guangzhou, and they also failed to detect the presence of virus using a TaqMan MGB real-time PCR method. In contrast, many studies from other countries have reported a high infective rate of dengue virus in wild-derived *Aedes* mosquitoes. For example, the minimum infective rate (MIR) in *Ae. albopictus* and *Ae. aegypti* collected in Singapore, from April 1995 to July 1996, was 50 and 57.6, respectively.[56] Kow

and others[57] reported that they used a single-step RT-PCR to assay the male vector (adults) collected from the field of Singapore and found that 1.33% and 2.15% of *Ae. aegypti* and *Ae. albopictus*, respectively, were positive for dengue viruses. The serotypes detected in male *Ae. aegypti* were DV1 (44%), followed by DV2 (22.2%) and DV3 (22.2%) and DV4 (11.1%). In *Ae. albopictus* males, the serotypes were DV4 (38.9%), followed by DV2 (33.3%), DV3 (16.7%), and DV1 (11.1%). These results emphasize the need for sensitive methods for the identification of the viruses that would allow us to address questions about the presence and absence of infectious virus, the origins of the strains in specific outbreaks, and whether viral genotypes in one epidemic are related to those causing previous or subsequent epidemic.

PREVENTION AND CONTROL

In mainland China, the primary goals of DF control should be timely epidemic detection, prevention, and control of new cases to prevent large-scale outbreaks and to minimize the damage of the epidemic. On the basis of the global DF/DHF prevention and control strategies drafted by WHO in 1995,[2] and considering the actual domestic condition, China has instituted its DF control strategy with close monitoring as the key, accompanied by control of mosquito vector density, high vigilance for imported cases, improvement of diagnosis and treatment, enhancing the cooperation among the related different public health departments, and mobilizing the social effort to combat the epidemic.

Mosquito vector monitoring. The DF is characterized by periodical and sudden occurrence with rapid transmission, often resulting in an unexpected large-scale epidemic.[7] A sensitive monitoring system that allows prediction and early detection of an epidemic is critical to the reduction and arresting of the epidemic. This monitoring system involves examination of vector species, their distribution, breeding places, density, sensitivity to pesticides, and viral subtype identification. China currently monitors the major vector mosquitoes of the genus *Aedes*, primarily by examining mosquito larvae using the Breteau index, house index, and container index. This approach has produced encouraging results in the high-risk regions for DF (such as Hainan and Guangxi).[43,58,59] However, the larval and pupal density of mosquitoes in a defined environment may not represent the exact density of the adult mosquitoes, therefore such an approach can be laborious and the results subjected to influences by various factors. Because *Ae. albopictus* is generally not domestic, Lin and others[60] designed a mosquito trap based on the U.S. Centers for Disease Control and Prevention (CDC, Atlanta, GA) trap. This new trap allows close observation of the seasonal variation in the number of adult *Ae. albopictus*, and complete data sets on mosquito densities in the larval, pupal, and adult stages. Adult mosquitoes trapped also are useful as specimens for virus detection, which can be of great significance in the prediction and control of an DF outbreak.[61–63] The preliminary testing results of this new mosquito trap showed its greater advantages over the Breteau index for *Ae. albopictus* monitoring in the urban areas.[64]

Vector control. Because no DF vaccine is currently available, the only effective means for DF prevention is mosquito vector control. The key to this control relies on the maintenance of environmental hygiene and elimination of the breeding places of the mosquitoes.[65] Pesticide application remains the conventional means for eliminating the vectors in the breeding sites. However, these activities are associated with the emergence of pesticide resistance and this is evident already in some regions. The use of pesticides is by no means an environmentally friendly solution to the mosquito problem. More environmentally friendly methods therefore are urged for controlling the breeding places of the mosquitoes. Some cost-effective biocontrol agents such as *Bacillus thuringiensis* var. *israelensis* and *predatory cyclops* that do not cause environmental damage or produce resistance in the mosquitoes after long-term use are promising.[66,67] However, these slow-acting agents may not seem appropriate in emergency settings,[66] and pesticides are still necessary for mosquito control during an DF or DHF outbreak. The WHO[68] recommends the ultra-low volume (ULV) spray or thermospray of malathion and sumithion to eliminate the mosquitoes around breeding sites. However, because of its poor effect against female mosquitoes and the larvae,[69,70] UVL aerosol is seldom used for outdoor mosquito control during the epidemic of DF or DHF.[68]

FUTURE CHALLENGE

Accelerated urbanization, expanding urban populations, frequent international travel, and perhaps global warming, all may contribute to increasing the frequencies of DF/DHF epidemics. The absence of effective vaccines and robust measures for the vector management make DF and DHF control difficult, and they will remain a major public health issue in the tropical and subtropical regions. The challenges in DF control depend on the following four aspects: 1) vector control; much effort is needed to establish a monitoring system for early prediction of the occurrence and distribution of the mosquitoes on the basis of their chemical, biological and environmental control. Chinese researchers[71–73] have made preliminary but meaningful attempts at DF control using a geographic information system (GIS),[74] which allows prediction of the occurrence and distribution of the vectors as well as estimation of the probability of DF epidemic. 2) The establishment of a rapid virus detection system to provide early prediction of the epidemic, which necessitates effective international communication and cooperation. At the same time, the deciphering of the genome of *Aedes* mosquito[75] and the application of transgenic technology[76,77] have provided many platforms for dengue prevention and control. 3) Community-based education also is critical. The full knowledge of DF and dengue viruses among the community residents may help greatly to achieve the goal of controlling the monitoring indices within the safe limits, and the participation by the community can be of great importance in winning the battle against vector mosquitoes. 4) Elimination of the technical obstacles to develop safe and effective vaccines. Rapid progress is being made in research of the immune and pathogenic mechanisms of dengue fever, and better understanding of these mechanisms will facilitate the development of the safe and effective vaccines against dengue virus.

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別紙様式第2-1

No. 20

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
				2010. 10. 4	該当なし	

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販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		ProMED 20100927.3506, 27-SEP-2010.	フィリピンほか

研究報告の概要

○デング熱更新情報
・フィリピン
2010年1月1日から9月18日までのIloilo市における症例数は4,825例に達し、死亡が27例であった。全症例のうちIloilo市の12病院に限定した患者数は8月19日から9月までで32名であった。
・タイ
2010年1月から9月11日までの患者数は75,852例で、死亡が87例であった。ほとんどの患者は北東部で認められ、ついで中部、南部及び北部地域で認められた。国内の関連機関は各地域内での疾患の発症を管理するため、デング熱に対する予防措置に関して住民に指導するよう命じられた。
・マレーシア
死亡者数が昨年の同時期の70名に比べ今年は107名に増加し、死亡率は53%に上昇した。副首相は、疾患を一掃するために遺伝子組換えを行った蚊を放つ計画について、冷静な姿勢を示した。
・インド・デリー
首都圏で患者が急増しており、2010年9月26日に95例以上の症例が報告された。
・プエルトリコ
2010年の死亡例は23例であると2010年9月25日に発表された。8月27日から9月2日の週に新たに835例の症例が報告された。
・パラグアイ
2010年の疑い症例数が21,443例であり、確定症例数が13,678例であることを明らかにした。保健省は国民に対してネッタイシマカの発生源を排除するよう呼びかけている。

報告企業の意見	今後の対応
世界各地におけるデングウイルス感染状況の最新情報である。	日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を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

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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

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Archive Number 20100927.3506
Published Date 27-SEP-2010
Subject PRO/EDR> Dengue/DHF update 2010 (50)

DENGUE/DHF UPDATE 2010 (50)
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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:
Asia
[1] Philippines (Iloilo)
[2] Taiwan (Kaohsiung City)
[3] Viet Nam
[4] Thailand
[5] Indonesia (Jakarta)
[6] Malaysia
[7] China (Hong Kong)
[8] Nepal
[9] India (Delhi)
[10] India (Bihar)
[11] Pakistan (Kerachi)
Oceania
[12] Australia (north Queensland)

Europe
[13] Monaco ex Caribbean
[14] France (Corsica)
[15] France (Alpes Maritimes)

Americas
[16] Puerto Rico
[17] Mexico (Oaxaca)
[18] Honduras
[19] Brazil (Roraima)
[20] Brazil (Sao Paulo)
[21] Paraguay

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[1] Philippines (Iloilo)
Date: Wed 22 Sep 2010
Source: The Star [edited]
<http://www.thenewstoday.info/2010/09/22/iloilo.dengue.cases.hit.4.825.with.27.

Dengue cases in Iloilo province have now reached 4825 with 27 deaths as reported by the Iloilo Provincial Health Office from 1 Jan - 18 Sep 2010. Of the total dengue cases, the number of patients confined in the 12 provincial and district hospitals in Iloilo for this month [September 2010] alone starting 19 Aug [2010] is 32.

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<promed@promedmail.org>

[The numbers of cases and deaths in affected municipalities are given in the above URL.

The 25 Sep 2010 edition of the Macau DAily Times
(<http://www.macaudailytimes.com.mo/asia-pacific/17224-Philippines-Pools-the-ri
[2009].

A map showing the provinces in the Philippines can be accessed at <http://en.wikipedia.org/wiki/Provinces_of_the_Philippines>.
A HealthMap/ProMED-mail interactive map of the Philippines can be accessed at <http://healthmap.org/r/00bk>. - Mod.TY]

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[2] Taiwan (Kaohsiung City)
Date: Sun 26 Sep 2010
Source: RTI [edited]
<http://english.rti.org.tw/Content/GetSingleNews.aspx?ContentID=110467&BlockID=

Concern grows over the worsening dengue situation in Kaohsiung City. That was the word from Health Minister Yaung Chih-liang on Sunday [26 Sep 2010]. Dengue fever is raging in Kaohsiung, with 227 cases reported so far this year [2010]. Yaung is visiting the southern city to review the epidemic prevention work there. Yaung said that in addition to the indigenous cases in the city, there has been an increase in the number of imported cases of dengue.

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[A map of Taiwan can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/taiwan_pol92.jpg>. A HealthMap/ProMED-mail interactive map showing the location of the island of Taiwan can be accessed at <http://healthmap.org/r/00tD>. - Mod.TY]

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[3] Viet Nam
Date: Tue 21 Sep 2010
Source: Vietnam Plus [edited]
<http://en.vietnamplus.vn/Home/Prime-Minister-calls-for-action-on-dengue-fever/

Prime Minister Nguyen Tan Dung has instructed health officials to take firm measures to combat dengue fever, which has claimed 42 lives so far this year [2010] out of 55 400 cases reported nationwide. He urged the Ministry of Education and Training to mobilise pupils nationwide to join campaigns to kill mosquitoes -- the main carrier of the disease [virus] -- in their houses and public places.

Ha Noi reported that the disease had hit 27 out of the city's 29 districts. In August 2010 alone, the whole country recorded 19 577 patients and 11 deaths in 54 cities and provinces, according to reports from the Preventive Medicine Department.

The number of patients hospitalised in HCM City in the 1st 2 weeks of this month [September 2010] increased 30 percent, in comparison with the same period in previous months. Children's Hospital No 2 reported that it had received 555 dengue fever patients since the start of this month, and on average was receiving between 15-30 patients a day.

The preventive medicine department said it was working with relevant agencies to take preventive measures in key localities since early this year [2010], focusing on cleaning the environment and killing mosquitoes.

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[A map of Viet Nam with provinces can be accessed at <http://upload.wikimedia.org/wikipedia/commons/f/f0/VietnameseProvincesMap.png> Mod.TY]

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[4] Thailand
Date: Sat 18 Sep 2010
Source: National News Bureau of Thailand (NNT), Public Relations Department [edited]
<http://thainews.prd.go.th/en/news.php?id=255309180018>

Public Health Minister Jurin Laksanawisit stated that from January - 11 Sep 2010, the numbers of patients infected by dengue fever were at 75 852 with 87 deaths. Most patients were found in Northeastern provinces, followed by the Central, Southern and Northern regions.

However, the Minister said the number of dengue fever patients in the South was already reduced, but the North and Northeast still needed to be closely monitored. The Ministry has also instructed all related agencies to report the progress of problem-solving continuously.

Relevant agencies nationwide are ordered to educate the people on preventive measures against dengue fever to control disease outbreaks in each area.

[Byline: Panita Norasing]

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<promed-mbds@promedmail.org>

[According to the Thai Ministry of Public Health, Bureau of Epidemiology's (BOE) report of the situation of dengue infection in Thailand, between 1 Jan 2010 and 10 Sep 2010, available in Thai at <http://epid.moph.go.th/dhf/situation/y53/Dengue_10Sep2010.pdf>, a total of 75 852 cases and 87 deaths were reported nationwide. The attack rate was 119.53 per 100 000 population. The case fatality rate was 0.11 percent. The highest percentage of cases was reported in the 15-24 years old age group (27.5 percent), followed by the 10-14 years old age group (25.1 percent), 5-9 years old age group (16.7 percent) and 25-34 years old age group (11.8 percent).

In the last 4 weeks, the 10 provinces with the highest attack rates of dengue infection (per 100 000 population) were Phayao (65.9), Chiang Mai (51.0), Songkhla (31.6), Narathiwat (31.5), Phatthalung (31.3), Petchabun (27.6), Rayong (27.1), Tak (26.9), Lopburi (25.3) and Surin (24.8).

Maps of Thailand showing the distribution of cases in the last 4 weeks are available on the last page of the report. In the map, the red color represents provinces that reported cases with an attack rate of 10.01 per 100 000 population and above; yellow represents provinces that reported cases with an attack rate between 5.01 and 10.00 per 100 000 population, and green, provinces that reported cases with attack rates between 0.01 and 5.00 per 100 000 population.

For maps showing Thailand's provinces, see <http://www.thailand-map.net/thailand_provinces/> and regions, see <http://thailandforvisitors.com/general/regions.html>.
For the interactive HealthMap/ProMED-mail map of Thailand with links to other recent ProMED-mail and PRO/MBDS postings, see <http://healthmap.org/r/00cC>. - Mod.SCM]

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[5] Indonesia (Jakarta)
Date: Sun 26 Sep 2010
Source: Berita Jakarta [edited]
<http://www.beritajakarta.com/2008/en/newsview.aspx?idwil=0&id=16741>

It seems that dengue fever is still a serious threat for Central Jakarta residents, especially with the transition weather happening in the last few days. Evidently, the number of dengue cases in Central Jakarta area from January-September this year [2010] reached 1565 cases .... and from 1-20 Sep 2010, 260 dengue cases have been recorded.

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[Dengue cases in specific sub-districts can be seen at the above URL.

A map of Indonesia showing the location of Jakarta in Banten province on Java Island can be accessed at <http://www.lib.utexas.edu/maps/middle_east_and_asia/indonesia_pol_2002.jpg>.
A HealthMap/ProMED-mail interactive map of Indonesia can be accessed at <http://healthmap.org/promed/en?v=-2.6,120.9,5>. - Mod.TY]

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[6] Malaysia
Date: Wed 22 Sep 2010
Source: Arab Times [edited]
<http://www.arabtimesonline.com/NewsDetails/tabid/96/smid/414/ArticleID/159843/

Malaysia said Monday [20 Sep 2010] its dengue fever death rate spiraled 53 percent this year [2010], but backed away from a controversial trial of releasing genetically modified mosquitoes to wipe out the disease.

"There was a major rise in deaths due to dengue fever, with 107 deaths so far this year [2010] compared to 70 deaths for the same period last year," said deputy premier Muhyiddin Yassin.

Muhyiddin said the majority of the deaths could have been avoided, and urged the public to take action to eradicate the _Aedes aegypti_ mosquitoes -- which spread dengue [viruses] -- from their homes and workplaces. "We have identified 19 hotspots throughout the country where the disease is prevalent, and action is being taken to tackle the situation in these areas," he said.

However, Muhyiddin was cool on a plan to release genetically modified male mosquitoes designed to combat dengue fever, in a proposed landmark field trial that has come in for criticism from environmentalists. In the 1st experiment of its kind in Asia, 2000-3000 male _Aedes aegypti_ mosquitoes were to be released in 2 Malaysian states in October or November 2010 if the plan had won government support. The insects in the study have been engineered so that their offspring quickly die, curbing the growth of the population in a technique researchers hope could eventually eradicate the dengue mosquito altogether. Muhyiddin said the project would not be implemented "at the moment." The field trial for the GM anti-dengue mosquitoes was developed by a British-based insect bio-tech company, Oxitec, and was to be undertaken by Malaysia's Institute for Medical Research, an agency under the health ministry.

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[It appears that Oxitech is applying the sex-specific alternative splicing in insects to engineer female-specific autocidal genetic systems developed in the Mediterranean fruit fly, _Ceratitis capitata_ to _Aedes aegypti_. It will be interesting to see what the results are of this approach to dengue vector control if and when field trials are carried out. Success would likely depend on the released, genetically modified male mosquitoes being able to survive in nature and compete with wild males for mating with wild females.

Reference:
Fu G, Condon KC, Epton MJ, Gong P, Jin L, Condon GC, Morrison NI, Dafa'alla TH, Alphey L. 2007. Female-specific insect lethality engineered using alternative splicing. Nat Biotechnol 25(3):353-357.

A HealthMap/ProMED-mail interactive map of Malaysia can be accessed at <http://healthmap.org/r/009L>. - Mod.TY]

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[7] China (Hong Kong)
Date: Thu 23 Sep 2010
Source: MB.com.ph [edited]
<http://www.mb.com.ph/articles/278536/hong-kong-reports-first-local-case-denque

A case of locally-acquired dengue fever, involving a 46-year-old man, was confirmed in Hong Kong, which was the 1st local [locally acquired] case in 7 years, the Center for Health Protection under the city's Department of Health said Wednesday [22 Sep 2010]. A spokesman with the Center for Health Protection said the Department of Health was highly concerned about this case of locally-acquired dengue fever infection since the last local dengue fever outbreak in Hong Kong occurred in 2002. "We are working with the Food and Environmental Hygiene Department closely to make an all-out effort to assess if there is any spread of the infection, contain the infection, and

prevent the spread," he said.

A total of 61 cases of dengue fever [60 imported] has been reported to the Center for Health Protection in Hong Kong so far this year [2010]. There were 43 cases in 2009, and all of them were classified as imported.

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A map showing the location of Hong Kong China's east coast can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/china_pol01.jpg>.
A HealthMap/ProMED-mail interactive map of China can be accessed at <http://healthmap.org/r/00cv>. - Mod.TY]

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[8] Nepal
Date: Mon 20 Sep 2010
Source: Xinhua Net [edited]
<http://news.xinhuanet.com/english2010/health/2010-09/20/c_13521157.htm>

At least 19 persons have died in a month, and more than 7000 have been afflicted with dengue and viral fever in Chitwan in central Nepal, The Himalayan Times daily reported on Monday [20 Sep 2010]. According to Mahendra Prasad Shrestha, District Public Health Officer, 9 persons have died of dengue and viral fever in Chitwan Medical College (CMC), 6 in Bharatpur Medical College and 4 in Bharatpur Hospital since the spread of the diseases in the district in the last week of August 2010.

According to Dr Shital Adhikari, head of the Medical Department of CMC, 40 patients with dengue fever were visiting the hospital daily, and at least 12 of them had tested positive for dengue.

[Editor: Deng Shasha]

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<promed@promedmail.org>

[A HealthMap/ProMED-mail interactive map of Nepal showing the location of Kathmandu can be accessed at
<http://healthmap.org/promed/en?v=28.3,83.9,5>. - Mod.TY]

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[9] India (Delhi)
Date: Sun 26 Sep 2010
Source: The Times of India [edited]
<http://timesofindia.indiatimes.com/city/delhi/95-more-dengue-cases-in-Delhi-to

Dengue continues to surge in the national capital, with 95 more cases being reported on Sunday [26 Sep 2010], taking the total number of people infected with the mosquito-borne disease to 2916, an official said. This year, 5 people have succumbed to dengue in the capital, including one from outside Delhi, a Municipal Corporation of Delhi (MCD) official said.

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[Since patients coming to private clinics and hospitals may not be reported officially, this number is probably a significant under-estimate of the actual number of cases.

A HealthMap/ProMED-mail interactive map of India showing the location of Delhi can be accessed at <http://healthmap.org/r/01Jf>. - Mod.TY]

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[10] India (Bihar)
Date: Wed 22 Sep 2010
Source: Data News & Analysis [edited]

<http://www.dnaindia.com/india/report_dengue-death-toll-in-bihar-at-6-518-other

In the Munger district of Bihar, 6 people have died so far due to the outbreak of dengue, while 518 others have tested positive, official sources said today [22 Sep 2010]. Unofficial sources have, however, claimed that the number of casualties due to suspected mosquito-bred [transmitted] fever [virus] stands at 27. As many as 518 out of 1361 people diagnosed for symptoms of the dengue fever have tested positive, officials said.

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[A map of India showing the location of Bihar state in the far northeast can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/india_pol01.jpg>.
- Mod.TY]

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[11] Pakistan (Karachi)
Date: Thu 23 Sep 2030
Source: Daily Times [edited]
<http://www.dailytimes.com.pk/default.asp?page=2010\09\23\story_23-9-2010_pg12

Officials said the number of citizens affected with dengue virus has also increased in the province, as 13 new cases were reported during the last 24 hours; 9 of them were admitted [to hospital], while 4 were sent home, they added. According to the Provincial Dengue Surveillance Cell, the total number of dengue cases reported this year [2010] was 356, of which 216 people were tested positive; while one person, who was taken to a private hospital on 10 Sep [2010], died on 21 Sep [2010].

[Byline: Irfan Aligi]

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[The 26 Sep 2010 edition of The Business Recorder (<http://beta.brecorder.com/section/37/1/1106118:many-suffering-from-dengue-fev
city.

A map showing the locations of Sindh province and Karachi can be accessed at
<http://www.lib.utexas.edu/maps/middle_east_and_asia/pakistan_pol_2002.jpg>. A HealthMap/ProMED-mail interactive map of Pakistan can be accessed at
<http://healthmap.org/r/05Pd>. - Mod.TY]

******
[12] Australia (North Queensland)
Date: Fri 24 Sep 2010
Source: Sydney Morning Herald [edited]
<http://news.smh.com.au/breaking-news-national/dengue-outbreak-in-cairns-201009

An outbreak of dengue fever in Cairns has doctors concerned it could foreshadow a bad season for the mosquito-borne disease. 4 people from the suburb of Parramatta Park have tested positive to dengue, and another 8 are awaiting test results, Queensland Health medical director Dr Jeffrey Hanna said. More confirmed cases of dengue type 2 [virus infection] are expected, he said. And there are concerns the disease will be spread by travellers setting out from the far north Queensland city.

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[The 27 Sep 2010 edition of Couriermail.com.au (<http://www.couriermail.com.au/news/queensland/cairns-records-its-fifth-case-c
virus.

Maps showing the location of Cairns in Queensland can be accessed at <http://www.lib.utexas.edu/maps/australia/australia_pol99.jpg> and the HealthMap/ProMED-mail interactive map of Australia at <http://healthmap.org/r/03Bp>. - Mod.TY]

******
[13] Monaco ex Caribbean
Date: Fri 24 Sep 2010
Source: Maville.com [in French, trans. Corr.SB. summ., edited]
<http://www.monaco.maprincipaute.com/actu/actudet_--Monaco-Premier-cas-de-dengu

A young resident, aged 18, returned from the Caribbean with the disease. Since early September 2010, the government has been strengthening mosquito control.

"Monaco does not have any indigenous dengue cases," said Stephane Valeri, Government Counsellor for Social Affairs and Health. "However, we have identified a case of imported dengue fever in early September [2010]. There is nothing to worry about for this young 18 year old resident of Monaco, who returned from the Caribbean with the disease. He is now in perfect health," said Stephane Valeri.

However, with the announcement of the 1st indigenous dengue fever cases in Nice, mosquito control, already assiduous in gardens and public spaces, has been strengthened. The 1st objective is to kill the tiger mosquito larvae. "The tiger mosquito [_Aedes albopictus_. - JW] has been located in our area for 3 years now, says Philip Porcu, Territory Chief Technician, Directorate of Planning and Urban Development.

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[All it takes to initiate a dengue outbreak is the presence of a viremic individual in an area where there is a significant population of _Aedes_ mosquito vectors, as has been the case in nearby Nice, France this month (September 2010). The concern and vigorous preventive actions by Monaco health authorities are justified.

Although ProMED does not normally report imported dengue cases with no subsequent local transmission, the risk of transmission elicited this report.

A HealthMap/ProMED-mail interactive map of Monaco can be accessed at <http://healthmap.org/promed/en?v=43.7,7.4,5>. - Mod.TY]

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[14] France (Corsica)
Date: Tue 21 Sep 2010
Source: 24 Ore [in French trans. Corr.SB, edited]
<http://24ore.club-corsica.com/il-nous-rend-dengue_15043_2183.html#7>

Since the beginning of the season [2010], 3 people with symptoms of dengue have been reported on the island; 2 in Corsica, San Gavinu di Fiumorbu and Biguglia last week, and a 3rd in Corse-du-Sud, in a tourist. [These] 3 cases, called "imported," arose from a trip to Asia. As a precaution, the Vector and Mosquito Control services of the General Council of Haute-Corse conducted a chemical treatment around residential areas of the sufferers.

On the mainland, Paca (Province, Alpes, Cote d'Azur), 120 cases (non-indigenous) have been detected in 4.5 months. On Sunday [19 Sep 2010], the Ministry of Health confirmed the presence of a 2nd "indigenous" case in Nice . Hopefully, the tiger mosquito [_Aedes albopictus_. - JW] carrying the disease [virus] does not reach us [Corsica].

[Byline: Emmanuelle Peretti]

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[This is another location having a significant risk of local dengue

virus transmission.

A HealthMap/ProMED-mail interactive map of France showing the location of Corsica can be accessed at <http://healthmap.org/r/07GC>. - Mod.TY]

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[15] France (Alpes-Maritimes)
Date: Wed 22 Sep 2010
Source: Le Generaliste.fr [in French, trans. Corr.SB, edited]
<http://www.legeneraliste.fr
/layout/Rub_ACTU.cfm?espace=3DACTU&id_rubrique=3D101860&id_article=3D27003>

In addition to the 2 confirmed cases of indigenous dengue fever in a residential area west of Nice, there are thought to be more than "6-7 suspects" in the Maritime Alps, according to Xavier Lorre, departmental delegate of the Regional Agency.

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[See also the end of report [14] above. - Mod.JW]

[The 26 Sep 2010 edition of Le Progres.com (<http://www.leprogres.fr/fr/france-monde/article/3850667/Provence-apres-la-den week.

A HealthMap/ProMED-mail interactive map of France showing the location of Nice can be accessed at <http://healthmap.org/r/07GC>. - Mod.TY]

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[16] Puerto Rico
Date: 25 Sep 2010
Source: Google / EPA [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.google.com/hostednews/epa/article/ALeqM5geAK-Sr_eqRLNZ8hjJbMx93rHz6

The death of a 50-year-old man raised to 23 the number of dengue deaths in Puerto Rico so far this year [2010], according to a report issued today [25 Sep 2010] by the Department of Health. This report indicated that according to the Dengue Division of the [USA] CDC, during [epidemiological] week 35, which corresponds to the 27 Aug - 2 Sep [2010] period, 835 new dengue cases have been reported ... and the DHF cases remained at 28.

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[A HealthMap/ProMED-mail interactive map showing the location of Puerto Rico in the Caribbean can be accessed at <http://healthmap.org/r/018->. - Mod.TY]

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[17] Mexico (Oaxaca)
Date: Sun 26 Sep 2010
Source: ADN Sureste [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.adnsureste.info/index.php/notas-del-dia/18242-30-de-los-casos-de-de

The dengue outbreak remains at 1668 cases, of which 30 percent are hemorrhagic, although for now a reoccurrence of the outbreak cannot be considered to exist, nor have cases doubled, so that one hopes to continue in a more or less stable trend even with the contingency of increased rains.

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[The occurrence of 30 percent of DHF of the total dengue cases is unusually high. Either the classification of DHF cases is in error, or there is serious underreporting of non-DHF dengue cases.

A map showing the location of Oaxaca state in Mexico can be accessed at <http://www.lib.utexas.edu/maps/americas/mexico_pol97.jpg>. A HealthMap/ProMED-mail interactive map of Mexico can be accessed at <http://healthmap.org/r/04jP>. - Mod.TY]

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[18] Honduras
Date: 19 Sep 2010
Source: European Pressphoto Agency [in Spanish, trans. & summ. Mod.TY, edited]
<http://www.google.com/hostednews/epa/article/ALeqM5q_1tcZj5pU3GS1FXcLaVDCT5FZj

The Honduras Ministry of Health has registered at least 68 deaths from DHF this year [2010], said the head of the National Dengue Program, Roxana Araujo. Also, at least 60 258 classical dengue fever cases have been registered and 2276 of DHF, the official added. Araujo reiterated that the trend of disease incidence remains in decline in recent weeks, and the suspension of national emergency to combat [this disease] issued this past June 2010 is under study.

In 2009, DHF caused the deaths of at least 12 people, and in 2010, the greatest incidence of this disease has been registered in the past 15 years in Honduras, according to health authorities.

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[A HealthMap/ProMED-mail interactive map showing the location of Honduras in Central America can be accessed at <http://healthmap.org/r/072r>. - Mod.TY]

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[19] Brazil (Roraima)
Date: Fri 24 Sep 2010
Source: BV News [in Portuguese, trans. & summ. Mod.TY, edited]
<http://www.bvnews.com.br/cotidiano7149.html>

The total of confirmed dengue [virus] type 4 cases has increased to 12 in Roraima. The confirmation was sent from the Instituto Evandro Chagas (IEC) of Belem [national reference laboratory] after analysis of 70 samples.

Two technicians from the Ministry of Health are in Boa Vista to accompany actions to combat the vector [mosquito]. According to the chief of the State Nucleus for Dengue and Yellow Fever of the state Secretariat of Health, Joel Lima, despite the number of cases of dengue 4 [virus infection] not increasing, it is of concern, because the disease could spread with time. Due to this problem, health authorities decided to intensify action in Vila Central, with active searching for and elimination of breeding sites of the _Aedes aegypti_ mosquito.

As of now, there are 6383 confirmed cases of classical dengue fever in Roraima (including the 12 of dengue [virus] 4), 181 with complications and 77 with DHF. Last year [2009] from January - September 2009, 2878 cases were confirmed.

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[Dengue virus serotype 4 is now part of the national patrimony of dengue. It came to stay. - Mod.LJS]

[A map showing the states in Brazil can be accessed at <http://www.lib.utexas.edu/maps/americas/brazil.jpg>. A HealthMap/ProMED-mail interactive map of Brazil can be accessed at <http://healthmap.org/r/008S>. - Mod.TY]

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[20] Brazil (Sao Paulo)
Date: Wed 22 Sep 2010
Source: O Globo [in Portuguese, trans. & summ. Mod.TY, edited]
<http://oglobo.globo.com/cidades/mat/2010/09/22/dengue-atinge-4-100-pessoas-mat

Confirmed dengue cases reached 4100 people and killed 17 in Sao Vicente in Sao Paulo [state]. Although the worst period has passed, mosquito populations will increase more easily in the [upcoming] hot months, a continuing cause of concern. Last year [2009], for example, the municipal Secretariat of Health registered 44 cases of the disease, with no deaths since 2007.

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[There is a lengthy discussion of concerns and details of mosquito vector control activities at the above URL. - Mod.TY]

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[21] Paraguay
Date: Sat 25 Sep 2010
Source: Paraguay.com [in Spanish, trans. Mod.TY, edited]
<http://www.paraguay.com/nacionales/mas-de-13-mil-paraguayos-afectados-por-el-d

The Ministry of Public Health confirmed 13 678 dengue cases in the entire country this year [2010]. Health authorities issued a report calling for the populace to eliminate possible _Aedes aegypti_ mosquito breeding sites, since the [upcoming] hot season is ideal for the reproduction of this [virus] vector. The Health [Ministry] did not provide details of the origin of the cases but specified that of the 21 443 notifications [of suspected cases], 13 678 were confirmed.

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[Additional details are available at the above URL.

A map of Paraguay can be accessed at
. A
HealthMap/ProMED-mail interactive map of Paraguay can be accessed at
<http://healthmap.org/promed/en?v=-23.2,-58.4,5>. - Mod.TY]

[see also:
Dengue/DHF update 2010 (49) 20100921.3399
Dengue/DHF update 2010 (48) 20100915.3345
Dengue/DHF update 2010 (47) 20100913.3308
Dengue/DHF update 2010 (46) 20100906.3198
Dengue/DHF update 2010 (45) 20100830.3085
Dengue/DHF update 2010 (44) 20100826.3010
Dengue/DHF update 2010 (43) 20100819.2891
Dengue/DHF update 2010 (42) 20100817.2847
Dengue/DHF update 2010 (41) 20100810.2726
Dengue/DHF update 2010 (40) 20100805.2651
Chikungunya and dengue - France (02): risk 20100731.2564
Dengue/DHF update 2010 (30) 20100627.2152
Chikungunya and dengue - France ex overseas 20100616.2008
Dengue/DHF update 2010 (20) 20100426.1347
Dengue/DHF update 2010 (10) 20100304.0707
Chikungunya & dengue - India: (TN) conf. 20100212.0500
Dengue/DHF update 2010 (01) 20100104.0038]
.....mpp/sb/ty/msp/jw
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別紙様式第2-1

No. 17

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
				2010. 10. 4	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		ProMED 20100915.3343,15-SEP-2010, Source: The Clare Herald	アイルランド

研究報告の概要

○アヒル卵によるサルモネラ症、血清型ネズミチフス菌DT8－アイルランド
アヒル卵を摂取することによるサルモネラ症は計24例が報告され、アイルランドで近年記録された食中毒の中でも最大のものとなっている。感染者の年齢層は生後5ヶ月～80歳にわたり、全国的である。これを考慮してアイルランド当局では、2010年9月14日、アヒル卵の安全な摂取法に関する助言を行った。また、アヒル卵に触った後の手洗い等の衛生管理を継続することの重要性について警告している。
サルモネラネズミチフス菌DT8感染の症状は、嘔吐や下痢による軽度の症状から生命を脅かす疾患に変化してきている。乳児や妊婦、高齢者や病人は最も危険にさらされているため特に注意が必要である。
この食中毒の集団発生に鶏卵は関係していない。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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
アヒル卵の摂取によるサルモネラ症の症例数が計24例となり、アイルランドで近年に記録された食中毒発生の中で最大の原因となっているとの報告である。	日本赤十字社では、輸血による細菌感染予防対策として問診時に献血者の健康状態を確認し、発熱を伴う食中毒様の激しい下痢症状がある場合は1カ月間献血不適としている。また、全ての輸血用血液製剤について、保存前白血球除去及び初流血除去を導入している。さらに、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ほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

MedDRA/J Ver.13.1J

INTERNATIONAL SOCIETY FOR INFECTIOUS DISEASES

ProMED-mail

Back

Archive Number 20100915.3343
Published Date 15-SEP-2010
Subject PRO/AH/EDR> Salmonellosis, serotype Typhimurium DT8 - Ireland: duck eggs

SALMONELLOSIS, SEROTYPE TYPHIMURIUM DT8 - IRELAND: DUCK EGGS
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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: Tue 14 Sep 2010
Source: The Clare Herald [edited]
<http://www.clareherald.com/news/national/2010-more-confirmed-cases-in-food-p i

The investigation into the current outbreak of salmonellosis linked with the consumption of duck eggs is ongoing, with 5 new cases in August 2010. The latest confirmed cases brings the total number to date to 24 and it is now the largest food poisoning outbreak of salmonellosis recorded in recent years in Ireland.

In light of this, the Food Safety Authority of Ireland (FSAI) today, 14 Sep 2010, reiterated its advice on the safe consumption of duck eggs. The people infected have ranged from 5 months to 80 years of age. The latest cases tend to be linked with the consumption of duck eggs from small backyard flocks or private farms. The confirmed cases are nationwide. Hen eggs are not implicated in this outbreak.

The FSAI has advised to only consume duck eggs that have been thoroughly cooked and to cease using raw duck eggs in any dishes that will not be cooked thoroughly prior to eating. It also cautions on the importance of good hygiene practices being followed, such as washing hands and preparation surfaces after handling or using duck eggs.

Prof Alan Reilly, chief executive, FSAI said: "We are advising caterers, retailers, and consumers to treat duck eggs in the same way as they would raw chicken. We all know that we should never eat raw chicken. This is a risk that is well understood by everyone, both in terms of ensuring it is cooked thoroughly and also by maintaining good hygiene practices, thereby preventing cross contamination between raw food and ready-to-eat food."

He continued: "However, people may have forgotten that duck eggs have been associated with _Salmonella_ in the past and therefore, are not taking the correct precautions today. The fact that the outbreak is ongoing, underlines the huge importance attached to maintaining stringent hygiene practices when handling raw duck eggs. Even when duck eggs look clean, they may still have salmonellae on the outside of the shell and sometimes carry it on the inside of the egg."

"The symptoms of _Salmonella [enterica_ serotype] Typhimurium DT8 infection vary from mild discomfort due to vomiting and diarrhea, to life threatening illness. Infants, pregnant women, the frail elderly, and the sick are most at risk from food poisoning. Anyone who may have these symptoms and suspects it may have been from recently eating duck eggs should contact their doctor for advice," added Prof Reilly.

The FSAI is working in collaboration with the Department of Agriculture, Fisheries and Food on control measures for commercial flocks and also smaller backyard flocks on private farms. A code of practice has been published for commercial flock owners and also guidelines have been published for producers of small quantities of

duck eggs from backyard flocks. Work is also underway by Bord Bia [Irish Food Board] to develop a new quality assurance scheme to ensure a safe source of duck eggs in the future.

The FSAI is continuing to work closely with the Health Protection Surveillance Centre; the Department of Agriculture, Fisheries and Food; and various local authorities to control this outbreak and to prevent further cases of illness.

[Byline: Mike Fanning]

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[Duck eggs are larger than hen eggs and richer in flavor, lending a creamy depth to baked dishes. The shells of duck eggs are slightly tougher to crack. The tougher shell gives duck eggs a longer shelf life, about 6 weeks in the refrigerator, inside is a yolk that is larger in proportion to the white than that of a chicken egg.

We commonly associate raw or poorly cooked chicken eggs (alone or in recipes) with a risk of salmonellosis acquisition. The risk is also present for the eggs of other fowl such as ducks (as shown in this report), ostriches, and quail.

Illustrative references include
1. Miller AA: Human Salmonella typhimurium infection due to duck eggs. Brit Med J. 1952; 2(4776): 125-8; available at <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2021319/>.
2. Stokes R: Salmonella food poisoning traced to duck eggs. Ir J Med Sci. 1959; 34: 481-501.
3. Baker RC, Qureshi RA, Sandhu TS, Timoney JF: The frequency of salmonellae on duck eggs. Poult Sci. 1985; 64(4): 646-52; abstract available at <http://www.ncbi.nlm.nih.gov/pubmed/4001051>.
4. Erdogrul OT, Ozkan N, Cakiroglu E: Salmonella Enteritidis in quail eggs. Turk J Vet Anim Sci 2002; 26: 321-3; available at <http://journals.tubitak.gov.tr/veterinary/issues/vet-02-26-2/vet-26-2-20-0103-Mod.LL]

[The HealthMap/ProMED-mail interactive map of Ireland is available at <http://healthmap.org/r/00bn>. - Sr.Tech.Ed.MJ]

[see also:
Salmonellosis, serotype Enteritidis - USA (07): eggs 20100910.3276
Salmonellosis, serotype Enteritidis - USA: eggs, alert, recall 20100817.2846
Salmonellosis, restaurant - USA: (CO), undercooked eggs 20100805.2653
2008

Salmonellosis, eggs - Australia: (NSW) 20080104.0048
2007

Salmonellosis, eggs - Australia: (NSW) 20071229.4171
Salmonellosis, eggs - Australia (QLD) (03) 20070308.0821
Salmonellosis, eggs - Australia (QLD): recall 20070303.0749
2005

Salmonellosis, raw eggs - Australia (TAS) 20051209.3556
2004

Salmonella, eggs - UK ex Spain 20041019.2835]
.....ll/mj/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 2010年10月29日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	MMWR. 2010;Dispatch/59	公表国 ハイチ	
販売名（企業名）	別紙のとおり					

研究報告の概要

問題点：ハイチでコレラのアウトブレークが発生し、2010年10月27日時点で303名の死亡が報告されている。

ハイチにおいてコレラのアウトブレークが発生しており、2010年10月21日にハイチのNational Laboratory of Public Health of the Ministry of Public Health and Populationによって Viblio cholerae O1-serotype Ogawa-biotype El Torが同定された。2010年10月27日現在で4,722人が発症し、この内303人の死亡が報告されている。ほとんどの症例が田舎の人口密集地であるArtibonite Departmentで報告されているが、首都のあるOuest Departmentにおいて可能性例が報告されており、感染の拡大を見せている。これまでハイチではコレラが流行したことはなく、国民はコレラへの感受性が高いと考えられる。

使用上の注意記載状況・その他参考事項等

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。

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別紙

一般的名称	①乾燥弱毒生風しんワクチン*、②乾燥弱毒おたふくかぜワクチン
販売名（企業名）	①乾燥弱毒生風しんワクチン*、②乾燥弱毒おたふくかぜワクチン「化血研」
報告企業の意見	コレラはコレラ菌で汚染された水や食物を摂取することで感染する代表的な経口感染症であると共に、重大な感染症である。今回の報告では、患者、死亡者共に短期に著しく増加し、拡大を続けていることが報告されている。当所製剤の安全性に対し、現時点で特段の対応が急がれるものではないが、その動向には注意しておく必要があると考える。 上記製剤の製造には、当所において国内献血血漿から製造した血漿分画製剤である「人血清アルブミン」を使用している。当所の人血清アルブミンの製造工程には、孔径約 0.2μm の無菌ろ過工程が導入されており、その効果はバクテリアチャレンジテストにより確認されている。また、当所の人血清アルブミンの製造工程には細菌より小型であるウイルスの除去を目的としたウイルス除去膜ろ過工程も導入されており、その効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第 1047 号、平成 11 年 8 月 30 日）」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。よって、上記製剤に使用している人血清アルブミンはコレラに対する安全性を確保していると考える。更に、これまでに上記製剤によるコレラへの感染報告例は無い。 以上の点から、上記製剤はコレラに対する安全性を確保していると考える。

*現在製造を行っていない

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Centers for Disease Control and Prevention

MMWR Morbidity and Mortality Weekly Report

Dispatch / Vol. 59 October 28, 2010

Cholera Outbreak — Haiti, October 2010

An outbreak of cholera is ongoing in Haiti. On October 21, 2010, toxigenic *Vibrio cholerae* O1, serotype Ogawa, biotype El Tor was identified by the National Laboratory of Public Health of the Ministry of Public Health and Population in Haiti. Identification of the isolate was confirmed by CDC. Antimicrobial susceptibility testing of selected *V. cholerae* O1 isolates conducted at the National Laboratory of Public Health and at CDC demonstrated susceptibility to tetracycline (susceptibility to this drug predicts doxycycline susceptibility), ciprofloxacin, and kanamycin; and resistance to trimethoprim-sulfamethoxazole, furazolidone, nalidixic acid, sulfisoxazole, and streptomycin.

As of October 27, a total of 4,722 cholera cases with onset during October 21–27 and 303 deaths had been reported in Haiti (*1*). Most cases have been reported from Artibonite Department (*1*), a rural but densely settled area with several small urban centers. In addition, probable cases have been identified elsewhere in Haiti, including Ouest Department, where the capital city of Port-au-Prince is located.

Cholera is transmitted through fecal contamination of water or food and causes an acute, severe, watery diarrhea that can result in hypovolemic shock and death if not treated with fluid replacement promptly. Epidemic cholera has not been reported previously from Haiti; the population is immunologically naïve and therefore highly susceptible to infection with *V. cholerae* (*2–4*). The outbreak appears to have spread from an initial concentration of cases in Artibonite Department. An international public health response, led by the Ministry of Public Health and Population and including technical support from the Pan American Health Organization, CDC, and other governmental and nongovernmental organizations, is under way. The emphasis of the response is on 1) minimizing mortality by using oral rehydration for most cases and intravenous rehydration for severely ill patients and 2) preventing infection by promoting water treatment, adequate sanitation and hygiene, and safe food preparation (*5*).

No cases of cholera in travelers from Haiti to the United States have been reported to CDC. Cholera is notifiable in all U.S. states and territories. Clinicians should promptly report known or suspected cases of cholera to state or local health departments. Health departments that identify suspected or confirmed cases of cholera in travelers who have arrived recently from Haiti should e-mail CDC at eocreport@cdc.gov. The potential for spread in the United States is low because U.S. water, sanitation, and food systems minimize the risk for fecal contamination of food and water.

CDC has provided prevention and treatment guidance for travelers to and from Haiti online (available at http://wwwnc.cdc.gov/travel/default.aspx). Health departments, especially in areas with large Haitian populations that might be more likely to include recent travelers to Haiti, should consider providing cholera information to clinicians. Clinicians serving Haitian populations should be aware of the recommendations for diagnosis and treatment.

More information on cholera, including recommendations for treatment, laboratory testing, and scientific publications, is available at http://www.cdc.gov/cholera. Further information regarding the outbreak in Haiti is available at http://www.cdc.gov/haiticholera.

Reported by

Ministry of Public Health and Population, Haiti. Pan American Health Organization. CDC.

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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	総合機構処理欄
				2010. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. Lancet Infect Dis. 2010 Sep;10(9):597-602. Epub 2010 Aug 10.	公表国 インド
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			

研究報告の概要

○インド、パキスタン、英国での新規抗生物質耐性メカニズムの出現:分子学的、生物学的ならびに疫学的研究

背景:New Delhi metallo-β-lactamase 1(NDM-1)によるカルバペネム耐性グラム陰性腸内細菌は、世界的に重大な健康問題となる可能性があるため、インド、パキスタン、英国の多剤耐性腸内細菌におけるNDM-1陽性率を調査した。

方法:インドの2つの中心地 — チェンナイ(南インド)、ハリヤーナー(北インド)で腸内細菌の分離株が研究され、英国の国立リファレンス研究所に参照された。抗生物質の感受性を評価し、カルバペネム耐性遺伝子bla_{NDM-1}の存在がPCRによって明らかになった。分離株はパルスフィールドゲル電気泳動法によってタイピングされた。プラスミドは、S1ヌクレアーゼ分解およびPCRタイピングによって分析した。英国患者については、インドまたはパキスタンへの渡航および最近の入院の有無を調査した。

所見:NDM-1産生分離株は、チェンナイ 44株、ハリヤーナー 26株、英国 37株、その他のインド、パキスタン地域で73株が確認された。NDM-1は、ほとんどが*Escherichia coli*(36株)と*Klebsiella pneumoniae*(111株)に見つかり、チゲサイクリンとコリスチンを除く全ての抗生物質に高度耐性があった。ハリヤーナーから分離した*K.pneumoniae*分離株は遺伝的に均一であったが、英国およびチェンナイの分離株は遺伝的多様性を示した。ほとんどの分離株は、プラスミド上にNDM-1遺伝子を有し、英国およびチェンナイ分離株では受容株に容易にプラスミドが伝達されたが、ハリヤーナーの分離株は伝達性がなかった。英国のNDM-1陽性患者の多くは、前年にインド、パキスタンへの渡航歴があるか、これらの国と関連があった。

結論:NDM-1が世界的な公衆衛生問題となる可能性は大きく、国際的共同調査が必要である。

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
インド、パキスタン、英国で、多剤耐性腸内細菌におけるNew Delhi metallo-β-lactamase 1(NDM-1)陽性率を調査したところ、NDM-1はほとんどが*Escherichia coli*や*Klebsiella pneumoniae*で見つかり、また英国のNDM-1陽性患者の多くは、インド、パキスタンへの渡航歴やこれらの国と関連があることが分かったとの報告である。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、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を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

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Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study

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Summary

Background—Gram-negative Enterobacteriaceae with resistance to carbapenem conferred by New Delhi metallo-β-lactamase 1 (NDM-1) are potentially a major global health problem. We

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investigated the prevalence of NDM-1, in multidrug-resistant Enterobacteriaceae in India, Pakistan, and the UK.

Methods—Enterobacteriaceae isolates were studied from two major centres in India—Chennai (south India), Haryana (north India)—and those referred to the UK's national reference laboratory. Antibiotic susceptibilities were assessed, and the presence of the carbapenem resistance gene bla_{NDM-1} was established by PCR. Isolates were typed by pulsed-field gel electrophoresis of XbaI-restricted genomic DNA. Plasmids were analysed by S1 nuclease digestion and PCR typing. Case data for UK patients were reviewed for evidence of travel and recent admission to hospitals in India or Pakistan.

Findings—We identified 44 isolates with NDM-1 in Chennai, 26 in Haryana, 37 in the UK, and 73 in other sites in India and Pakistan. NDM-1 was mostly found among *Escherichia coli* (36) and *Klebsiella pneumoniae* (111), which were highly resistant to all antibiotics except to tigecycline and colistin. *K pneumoniae* isolates from Haryana were clonal but NDM-1 producers from the UK and Chennai were clonally diverse. Most isolates carried the NDM-1 gene on plasmids: those from UK and Chennai were readily transferable whereas those from Haryana were not conjugative. Many of the UK NDM-1 positive patients had travelled to India or Pakistan within the past year, or had links with these countries.

Interpretation—The potential of NDM-1 to be a worldwide public health problem is great, and co-ordinated international surveillance is needed.

Funding—European Union, Wellcome Trust, and Wyeth.

Introduction

Bacteria from clinical and non-clinical settings are becoming increasingly resistant to conventional antibiotics. 10 years ago, concern centred on Gram-positive bacteria, particularly meticillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* spp. Now, however, clinical microbiologists increasingly agree that multidrug-resistant Gram-negative bacteria pose the greatest risk to public health. Not only is the increase in resistance of Gram-negative bacteria faster than in Gram-positive bacteria, but also there are fewer new and developmental antibiotics active against Gram-negative bacteria and drug development programmes seem insufficient to provide therapeutic cover in 10–20 years.

The increase in resistance of Gram-negative bacteria is mainly due to mobile genes on plasmids that can readily spread through bacterial populations. Standardised plasmid typing methods are enhancing our understanding of the host ranges of these elements and their worldwide distribution. Moreover, unprecedented human air travel and migration allow bacterial plasmids and clones to be transported rapidly between countries and continents. Much of this dissemination is undetected, with resistant clones carried in the normal human flora and only becoming evident when they are the source of endogenous infections. The CTX-M-15 extended-spectrum β-lactamase (ESBL) encoded by $bla_{CTX-M-15}$ was first reported in India in the mid-1990s. The gene jumped from the chromosome of its natural hosts, *Kluyvera* spp, to plasmids that have subsequently spread widely, establishing CTX-M-15 as the globally-dominant ESBL and the primary cause of acquired resistance to third-generation cephalosporins in Enterobacteriaceae.

Recent surveys have identified ESBLs in 70–90% of Enterobacteriaceae in India and, although these collections might be a biased sample, they do suggest a serious problem, making the widespread use of reserved antibiotics such as carbapenems necessary. Rates of cephalosporin resistance are lower in other countries but the growing prevalence of ESBL producers is sufficient to drive a greater reliance on carbapenems. Consequently, there is selection pressure for carbapenem resistance in Enterobacteriaceae, and its emergence is a worldwide public

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health concern since there are few antibiotics in reserve beyond carbapenems. Already *Klebsiella pneumoniae* clones with KPC carbapenemase are a major problem in the USA, Greece, and Israel, and plasmids encoding the VIM metallo-carbapenemase have disseminated among *K pneumoniae* in Greece.

We recently reported a new type of carbapenem resistance gene, designated bla_{NDM-1}. A patient, repatriated to Sweden after admission to hospital in New Delhi, India, was colonised by *K pneumoniae* and *Escherichia coli* with bla_{NDM-1} on plasmids of varying size, which readily transferred between bacterial strains in vitro. We sought molecular, biological, and epidemiological data on New Delhi metallo-β-lactamase 1 (NDM-1) positive Enterobacteriaceae in India and Pakistan and investigated importation of the resistance gene into the UK by patients returning from the Indian subcontinent.

Methods

Bacterial isolates

Isolates of bacteria were identified from Chennai and Haryana in India. UK isolates were identified from referrals to the Antibiotic Resistance Monitoring and Reference Laboratory by UK microbiology laboratories between 2003 and 2009. We also identified isolates from other sites around Bangladesh, India, and Pakistan.

Procedures

Bacteria were identified via the Phoenix automated phenotypic identification criteria (Becton Dickinson, Oxford, UK) or with API 20E strips (bioMerieux, Basingstoke, UK). Minimum inhibitory concentrations (MICs) and carbapenem resistance were established by microbroth dilution (Phoenix), British Society for Antimicrobial Chemotherapy (BSAC) agar dilution, or disc diffusion.

Modified Hodge (cloverleaf) test involving distorted carbapenem inhibition zones and imipenem-EDTA synergy tests by disc, or the MBL Etest (AB bioMérieux, Solna, Sweden) were used to screen for metallo-β-lactamase production. The presence of bla_{NDM-1} was established by PCR with specific primers targeting the gene. PCR and sequencing were used to identify other resistant genes (bla_{CMY-4} and $bla_{CTX-M-15}$) carried by the bacterial isolates.

Conjugational transfer of antibiotic resistance to the laboratory strain *E coli* J53 was done on blood agar without selection. After 18 h, the mixed cultures were washed from the plates, suspended in saline, and plated onto MacConkey agar containing sodium azide (100 mg/L) and meropenem (2 mg/L). Transconjugants were confirmed to have bla_{NDM-1} by PCR analysis. Plasmids were subsequently isolated and typed on the basis of their origins of replication, as described by Carattoli and colleagues.

Genomic DNA was prepared in agarose blocks and digested with the restriction enzyme XbaI (Roche Diagnostics, Mannheim, Germany). DNA fragments were separated by pulsed-field gel electrophoresis (PFGE) on a CHEF-DR III apparatus (Bio-Rad, Hercules, CA, USA) for 20 h at 6 V/cm at 14°C with an initial pulse time of 0·5 s and a final pulse time of 30 s. Dendrograms of strain relatedness were created with BioNumerics software.

Genomic DNA in agarose blocks was digested with the restriction enzyme S1 (Invitrogen, Abingdon, UK). DNA fragments were separated by PFGE as above. In-gel hybridisation was done with a bla_{NDM-1} probe labelled with ^{32}P (Stratgene, Amsterdam, Netherlands) with a random-primer method. Plasmid DNA bands that hybridised with bla_{NDM-1} were cut from the gel, purified, and typed as described by Carattoli and colleauges.

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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

From Chennai, 75 *E coli*, 60 *Klebsiella* spp, and six other Enterobacteriaceae resistant to carbapenems were isolated from 3521 (4%) Enterobacteriaceae analysed throughout 2009. Of these 141 carbapenem-resistant Enterobacteriaceae, 44 (19 *E coli*, 14 *K pneumoniae*, seven *Enterobacter cloacae*, two *Proteus* spp, one *Citrobacter freundii*, and one *Klebsiella oxytoca*) were NDM-1 positive (about 1% of all resistant isolates). During that same period, 47 carbapenem-resistant isolates (24%) of 198 from Haryana were identified; from these, 26 (13%) were positive for NDM-1, and all were *K pneumoniae*. The Indian isolates from Chennai and Haryana were primarily from community acquired urinary tract infections, pneumonia, and blood-stream infections. The age range was 4–66 years with a mean of 36 years (SD 20) and a female to male ratio of about two to one.

In the UK resistant isolates increased in both 2008 and 2009 (figure 1). Isolates with the NDM-1 enzyme, which was first detected in the UK in 2008, became the predominant carbapenemase-producing Enterobacteriaceae in 2009, accounting for 32 (44%) of 73 carbapenemase producers. During 2008–09, 37 Enterobacteriaceae isolates with the NDM-1 enzyme were referred from 25 laboratories across England with single representatives also from Scotland and Northern Ireland. These were identified as *K pneumoniae* (21 isolates), *E coli* (seven), *Enterobacter* spp (five), *Citrobacter freundii* (two), *Morganella morganii* (one), and *Providencia* spp (one). They were from 29 patients and had been isolated from urine (15 patients), blood (three), burn or wound swab (four), sputum (two), central line tip (one), throat swab (one), or unknown specimens (three). The mean age of the patients was 60 years (SD 24; range 1–87), with 17 male patients and 12 female patients. At least 17 patients had a history of travelling to India or Pakistan within 1 year, and 14 of them had been admitted to a hospital in these countries. Reasons for these admissions included renal or bone marrow transplantation, dialysis, cerebral infarction, chronic obstructive pulmonary disease, pregnancy, burns, road traffic accidents, and cosmetic surgery.

Isolates, NDM-1-positive bacteria from Mumbai (32 isolates), Varanasi (13), and Guwahati (three) in India, and 25 isolates from eight cities in Pakistan (Charsadda, Faisalabad, Gujrat, Hafizabad, Karachi, Lahore, Rahim Yar Khan, and Sheikhupura) were also analysed in exactly the same manner but in laboratories in India and Pakistan. These isolates were from a range of infections including bacteraemia, ventilator-associated pneumonia, and community-acquired urinary tract infections.

All the isolates producing the NDM-1 enzyme were resistant to several antibiotic classes (table). The 37 UK isolates were all resistant to imipenem and ertapenem, although a single *M morganii* isolate remained susceptible, at least in vitro, to meropenem (MIC 2 mg/L). Only four UK isolates remained susceptible to the monobactam aztreonam (MICs ≤1 mg/L), which is unaffected by metallo-carbapenemases including NDM-1; the other UK isolates were all resistant to all β-lactams, including aztreonam, suggesting the concurrent presence of additional β-lactamases including ESBLs and AmpC enzymes—identified by sequencing as mainly $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CMY\text{-}4}$. All 37 isolates were resistant to amikacin and tobramycin, although one isolate was susceptible to gentamicin and three to ciprofloxacin. MICs of minocycline were consistently 2 mg/L or greater, interpreted as non-susceptible with the BSAC and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for

doxycycline, but most (33 of 37) were susceptible to colistin (MICs ≤4 mg/L) and 26 were suseptible to tigecycline (MICs ≤1 mg/L; figure 2).

The 44 isolates from Chennai were similarly resistant to all β-lactam antibiotics, fluoroquinolones, and aminoglycosides, apart from two that were sensitive to gentamicin. 39 were resistant to minocycline with MICs >2 mg/L, 19 to tigecycline, and three to colistin (table and figure 2). Two of the three isolates resistant to colistin were *Proteus* spp, which are intrinsically resistant, and the third was a *K pneumoniae* strain (colistin MIC >32 mg/L; tigecycline MIC 8 mg/L). Although several reports from Greece have noted *K pneumoniae* isolates as colistin resistant, we believe our isolate is truly pan-resistant. Most of the 26 Haryana isolates were resistant to all β-lactam and non-β-lactam antibiotics, although three were susceptible to aztreonam and one to ciprofloxacin and amikacin. Minocycline MICs for the Haryana isolates were 8–16 mg/L and ten isolates had intermediate resistance (2 mg/L) to tigecycline by EUCAST criteria. None were resistant to colistin (table and figure 2).

The 21 *Klebsiella* isolates from the UK had different PFGE profiles and were typed to 19 distinct groups with only two related pairs, both of which included isolates from epidemiologically linked patients, probably representing cases of cross-infection. All the UK *E coli* isolates were different. The Chennai isolates were also very different, with none similar to each other. By contrast, the 26 NDM-positive *K pneumoniae* isolates from Haryana belonged to a single PFGE profile suggesting clonal spread. We could not prove statistically significant strain relatedness between the Indian and UK isolates.

Isolates from Chennai, Haryana, and the UK's Antibiotic Resistance Monitoring and Reference Laboratory were analysed for the location of the bla_{NDM-1} gene by S1 digestion of DNA, and then PFGE and direct probing of the gels with a radiolabelled bla_{NDM-1} gene. Each of the three groups of isolates typically carried several plasmids, with some isolates having up to eight plasmids (figure 3).

Indian isolates had bla_{NDM-1} exclusively on plasmids. Plasmids from the non-clonal Chennai isolates ranged from 50 kb to 350 kb, whereas those from the clonal *K pneumoniae* from Haryana were predominately either 118 kb (54%) or 50 kb (36%). The UK isolates had a more diverse range of plasmid sizes, 80 kb to greater than 500 kb. Three UK isolates also carried bla_{NDM-1} on their chromosome, suggesting in-situ movement of bla_{NDM-1}. There were many plasmids of identical size in isolates collected from India and the UK (data not shown), suggesting plasmid movement between bacterial isolates. In some isolates, bla_{NDM-1} was carried on more than one plasmid (figure 4).

47 isolates from Chennai (33) and Haryana (14) were randomly chosen for further investigation with PCR and DNA probing to verify the origin of replication (incompatibility type) for plasmids carrying bla_{NDM-1}. Plasmids carrying bla_{NDM-1} from the 14 isolates from Haryana could not be typed. 13 of the 33 isolates from Chennai carried bla_{NDM-1} on A/C-type plasmids and one bla_{NDM-1} positive plasmid was incompatibility type FI/FII. Similarly, when the 32 randomly selected UK isolates were assessed with the same methods, 22 carried A/C type plasmids. The other bla_{NDM-1} positive plasmids from India and the UK that were A/C and FI/FII negative could not be typed.

Transconjugants were created in *E coli* J53 from the 33 Chennai and 32 UK isolates; however, the isolates from Haryana did not produce transconjugates. All transconjugants were shown by PCR to contain bla_{NDM-1}. We compared the sizes of the plasmids in the clinical strains with those of the transconjugants and, in about 10% of cases, the plasmid had altered in size during transfer. In most cases the plasmid had lost DNA but two of 102 had gained DNA during transfer.

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In addition to the collections of isolates from Chennai and Haryana detailed above, we have confirmed by PCR alone the presence of genes encoding NDM-1 in carbapenem-resistant Enterobacteriaceae isolated from Guwahati, Mumbai, Varanasi, Bangalore, Pune, Kolkata, Hyderabad, Port Blair, and Delhi in India, eight cities (Charsadda, Faisalabad, Gujrat, Hafizabad, Karachi, Lahore, Rahim Yar Khan, and Sheikhupura) in Pakistan, and Dhaka in Bangladesh (figure 5) suggesting widespread dissemination.

Discussion

Enterobacteriaceae with NDM-1 carbapenemases are highly resistant to many antibiotic classes and potentially herald the end of treatment with β-lactams, fluoroquinolones, and aminoglycosides—the main antibiotic classes for the treatment of Gram-negative infections. Only a few isolates remained sensitive to individual aminoglycosides and aztreonam, perhaps owing to the loss of resistance genes (eg, those encoding aminoglycoside modifying enzymes, 16S rRNA methylases, or bla_{CMY-4}). Most isolates remained susceptible to colistin and tigecycline.

Typing did not identify common strain types of *E coli* or *K pneumoniae* between the Indian subcontinent and the UK or between north and south India. Nevertheless, the NDM-1-positive *K pneumoniae* isolates from Haryana were clonal, suggesting that some strains could potentially cause outbreaks. Most bla_{NDM-1} positive plasmids were readily transferable and prone to rearrangement, losing or (more rarely) gaining DNA on transfer. This transmissibility and plasticity implies an alarming potential to spread and diversify among bacterial populations. Curiously, many of the plasmids were incompatibility A/C types—a group not commonly associated with multidrug-resistant phenotypes.

Although antibiotic resistance in China has been highlighted as a concern, the rapid emergence of bla_{NDM-1} deserves equal attention. A recent editorial by Abdul Ghafur highlights the widespread non-prescription use of antibiotics in India, leading to huge selection pressure, and predicts that the NDM-1 problem is likely to get substantially worse in the foreseeable future. This scenario is of great concern because there are few new anti-Gram-negative antibiotics in the pharmaceutical pipeline and none that are active against NDM-1 producers. Even more disturbing is that most of the Indian isolates from Chennai and Haryana were from community-acquired infections, suggesting that bla_{NDM-1} is widespread in the environment.

The introduction of NDM-1 into the UK is also very worrying and has prompted the release of a National Resistance Alert 3 notice by the Department of Health on the advice of the Health Protection Agency. Given the historical links between India and the UK, that the UK is the first western country to register the widespread presence of NDM-1-positive bacteria is unsurprising. However, it is not the only country affected. In addition to the first isolate from Sweden, a NDM-1-positive *K pneumoniae* isolate was recovered from a patient who was an Australian resident of Indian origin and had visited Punjab in late 2009. The isolate was highly resistant and carried bla_{NDM-1} on an incompatibility A/C type plasmid similar to those in India and the UK.

Several of the UK source patients had undergone elective, including cosmetic, surgery while visiting India or Pakistan. India also provides cosmetic surgery for other Europeans and Americans, and bla_{NDM-1} will likely spread worldwide. It is disturbing, in context, to read calls in the popular press for UK patients to opt for corrective surgery in India with the aim of saving the NHS money. As our data show, such a proposal might ultimately cost the NHS substantially more than the short-term saving and we would strongly advise against such proposals. The potential for wider international spread of producers and for NDM-1-encoding plasmids to become endemic worldwide, are clear and frightening.

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Acknowledgments

TW, MT, and KK did MIC determinations, all genetic analysis, and plasmid profiling. Authors from HPA Centre for *Infections undertook strain typing, MIC determinations, and follow-up for UK cases. DP proofread the manuscript* and provided data from Australia. PK and MT (Chennai) and MS and UC (Haryana) characterised strains and provided clinical details. UR and AK provided prevalence data and demographics. All authors were involved in the compiling of the report and approved the final version.

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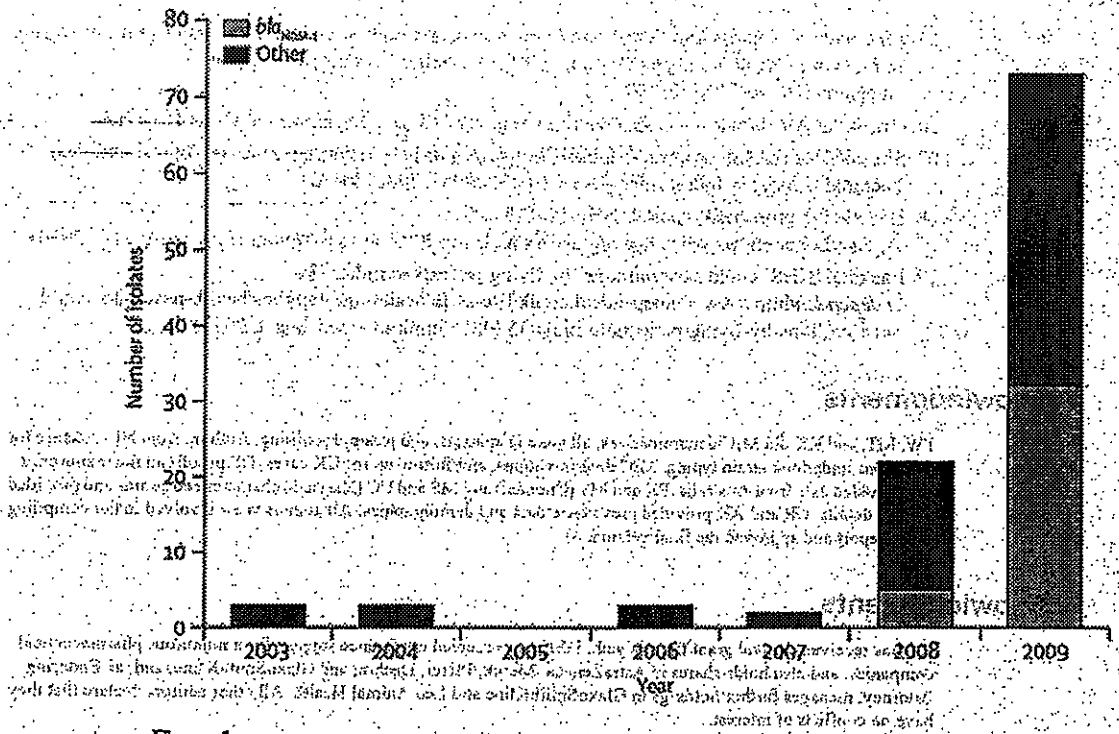

Figure 1.

Numbers of carbapenemase-producing Enterobacteriaceae referred from UK laboratories to the UK Health Protection Agency's national reference laboratory from 2003 to 2009

The predominant gene is bla_{NDM-1}, which was first identified in 2008. The other group includes diverse producers of KPC, OXA-48, IMP, and VIM enzymes.

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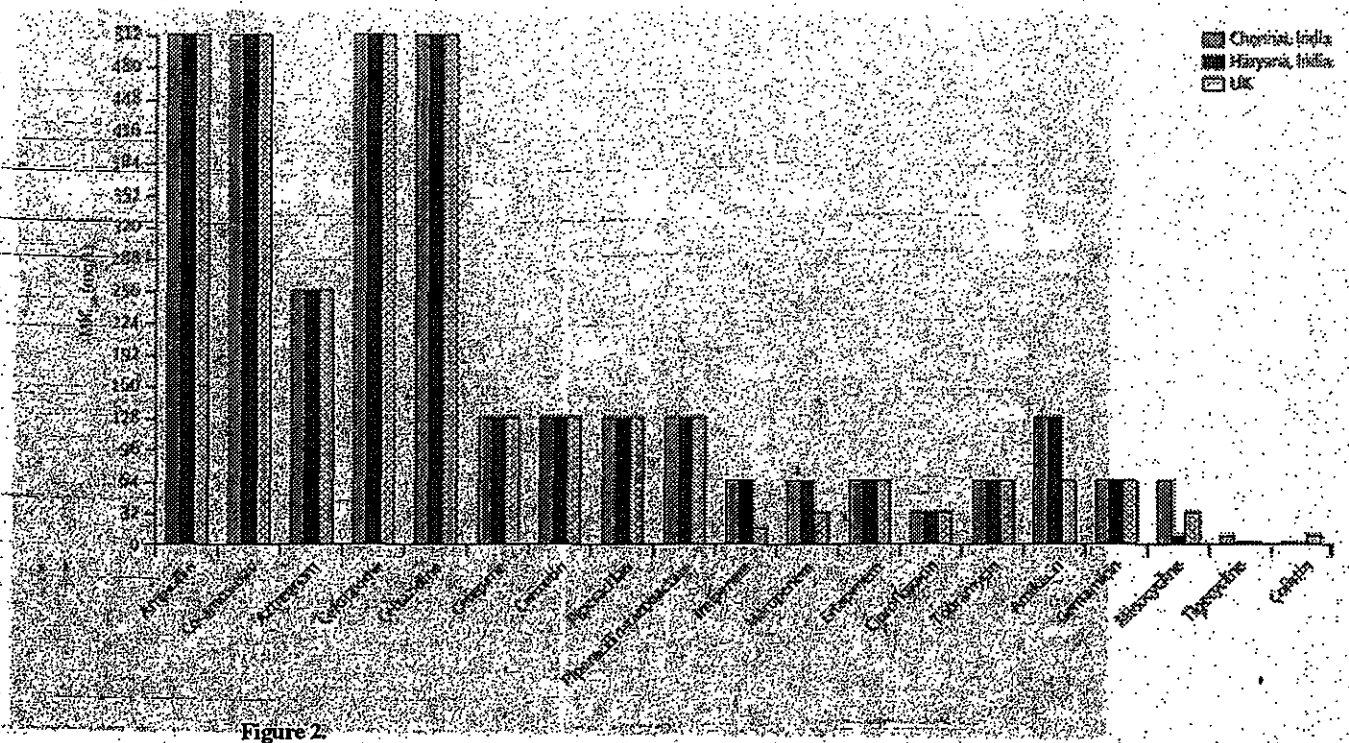

Figure 2:
90% minimum inhibitory concentration (MIC_{90}) for Enterobacteriaceae from Chennai and Haryana, India, and the UK

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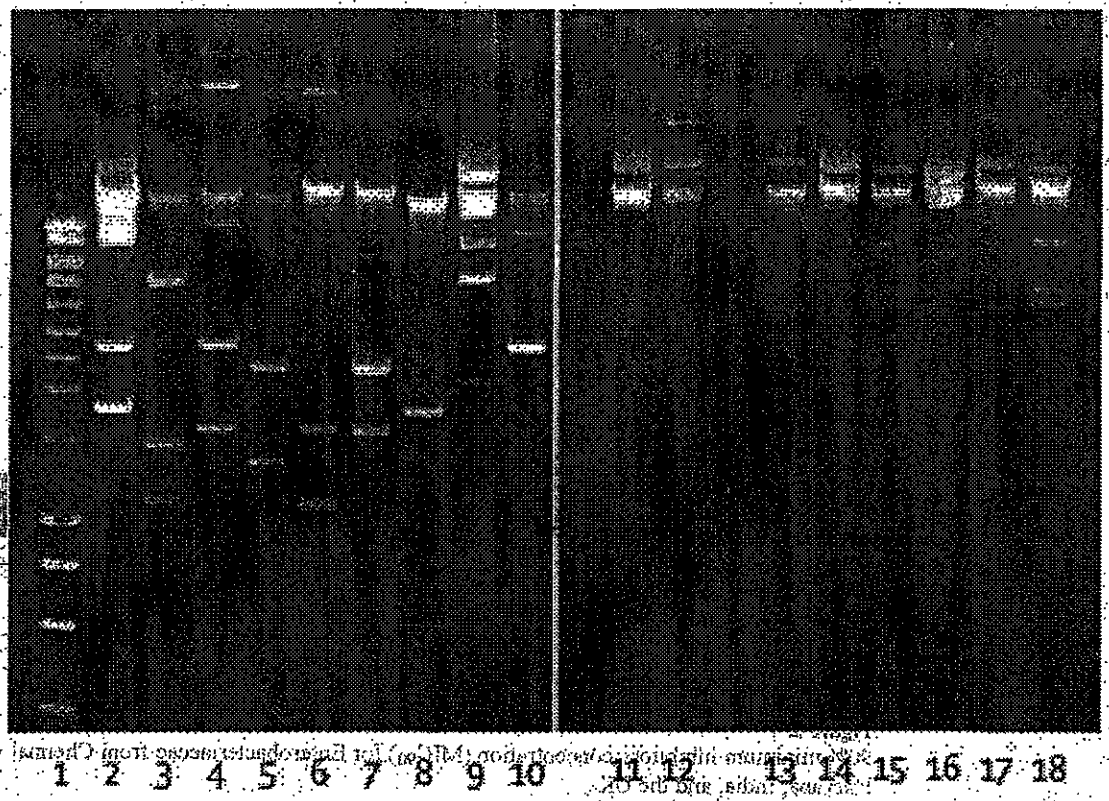

Figure 3.
The difference in plasmid numbers from a selection of Indian isolates
Tracks 1–10 show the number of plasmids in isolates from Chennai (south India) and tracks 11–18 show the number of plasmids in isolates from Haryana (north India). Most isolates contained up to seven plasmids, and in Chennai there was greater variation than in isolates from Haryana showing the bacterial clonality of NDM-1 carriage in Haryana.

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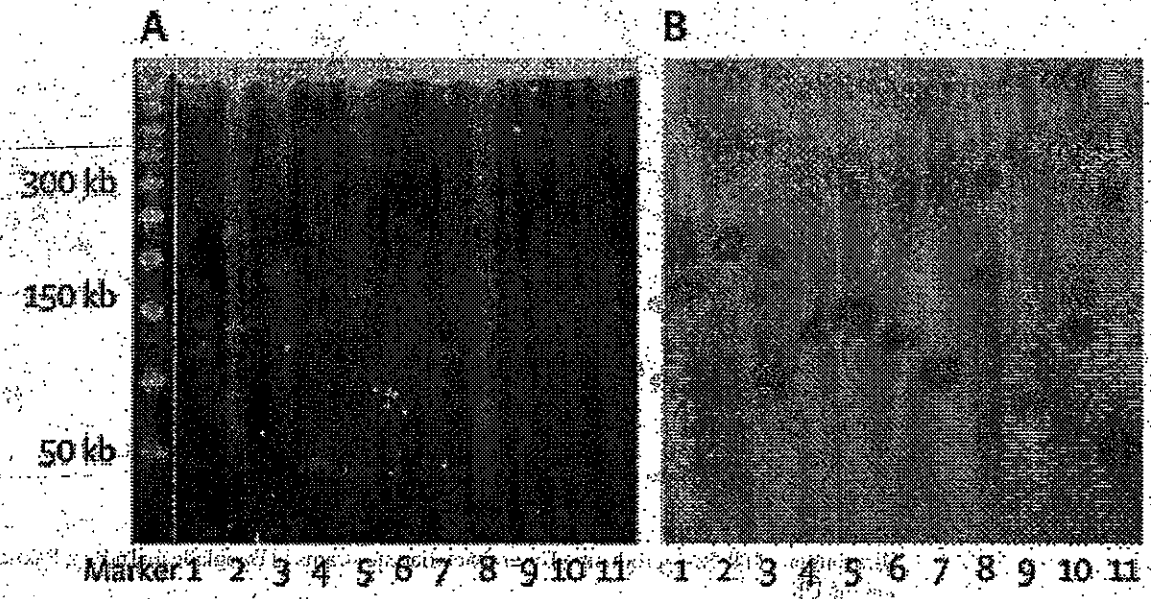

Figure 4.
Hybridisation results of UK isolates with bla_{NDM-1}
Pulsed-field gel of S1-treated plasmid DNA of UK isolates M15–M27 stained with ethidium bromide (A). Molecular weight marker is Lambda concatamer 50–1000 kb. The chromosome of each isolate is the bright band at the top of each lane and bright bands below are plasmids of various sizes. Autoradiogram of gel A probed with a bla_{NDM-1} showing individual or multiple plasmids in each strain carrying bla_{NDM-1} (B).

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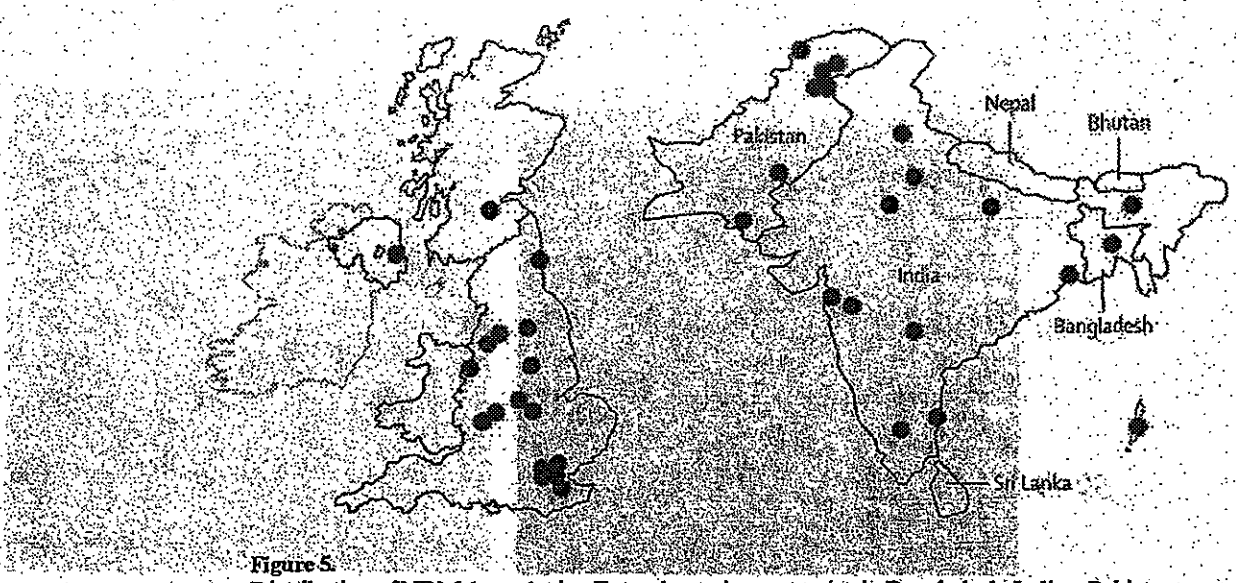

Figure 5.
Distribution of NDM-1-producing Enterobacteriaceae strains in Bangladesh, Indian, Pakistan, and the UK

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Table

Antibiotic susceptibilities for NDM-1-positive Enterobacteriaceae isolated in the UK and north (Chennai) and south India (Haryana)

	UK (n=37)		Chennai (n=44)		Haryana (n=26)	
	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*	MIC_{50}; MIC_{90} (mg/L)	Proportion susceptible*
Imipenem	32; 128	0%	64; 128	0%	32; 128	0%
Meropenem	32; 32	3%	32; >32	3%	>32; >32	3%
Piperacillin-tazobactam	>64; >64	0%	>64; >64	0%	>64; >64	0%
Cefotaxime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Ceftazidime	>256; >256	0%	>256; >256	0%	>256; >256	0%
Cefpirome	>64; >64	0%	>64; >64	0%	>64; >64	0%
Aztreonam	>64; >64	11%	>64; >64	0%	>64; >64	8%
Ciprofloxacin	>8; >8	8%	>8; >8	8%	>8; >8	8%
Gentamicin	>32; >32	3%	>32; >32	3%	>32; >32	3%
Tobramycin	>32; >32	0%	>32; >32	0%	>32; >32	0%
Amikacin	>64; >64	0%	>64; >64	0%	>64; >64	0%
Minocycline	16; >32	0%	32; >32	0%	8; 16	0%
Tigecycline	1; 4	64%	4; 8	56%	1; 2	67%
Colistin	0·5; 8	89%†	1; 32	94%†	1; 2	100%†

MIC=minimum inhibitory concentration.

*Susceptibility defined by British Society for Antimicrobial Chemotherapy and European Committee on Antimicrobial Susceptibility Testing breakpoints; doxycycline breakpoints were used for minocycline.

†Colistin-resistant UK isolates were one isolate of *Morganella morganii* and one *Providencia* sp (both intrinsically-resistant species), also one *Klebsiella pneumoniae* and one *Enterobacter* sp.

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別紙様式第2-1

No. 22

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
				2010. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	The Washington Times. Available from: http://www.washingtontimes.com/news/2010/sep/7/japan-confirms-its-first-case-of-new-superbug-gene/	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○日本初の超強力薬剤耐性菌症例
細菌を薬剤耐性菌に変化させる新たなNew Delhi metallo-β-lactamase 1(NDM-1)遺伝子が日本で初めて、インドで治療を受けた50歳代日本人男性に確認された。この遺伝子はほとんどすべての抗生物質に耐性となるよう細菌を変化させる。この遺伝子は主に病原性大腸菌で見られ、他のタイプの細菌に容易に複写され移入することが出来るDNA構造を有している。当該男性はインドで内科治療を受け、帰国後の2009年4月に入院した。男性がインドでどのような治療を受けたかについては、プライバシー保護のため公表されなかった。男性は入院中に高熱を出したが、2009年10月に退院した。病院は超強力薬剤耐性菌を疑い検体を保管、検査し、NDM-1遺伝子の検出について、厚生労働省に届け出た。院内感染は認められていない。日本初のNDM-1症例確認後、厚生労働省は全国調査を開始した。

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
細菌を薬剤耐性に変化させるNew Delhi metallo-β-lactamase 1遺伝子が、日本で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による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、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を喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

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The Washington Times

Japan confirms its first case of new superbug gene

By SHINO YUASA

Associated Press

3:01 a.m., Tuesday, September 7, 2010

TOKYO (AP) - Japan has confirmed the nation's first case of a new gene in bacteria that allows the microorganisms to become drug-resistant superbugs, detected in a man who had medical treatment in India, a Health Ministry offici said Tuesday.

The gene, known as NDM-1, was found in a Japanese man in his 50s, Kensuke Nakajima said.

Researchers say the gene _ which appears to be circulating widely in India _ alters bacteria, making them resistant t nearly all known antibiotics.

Drug-resistant bacteria are not new. Many bacteria are resistant to the world's first antibiotic, penicillin, as well as successive generations of drugs. Excessive use and improper use of antibiotics have exacerbated the problem and le to the emergence of superbugs.

"The potential of NDM-1 to be a worldwide public health problem is great, and coordinated international surveillan is needed," according to a widely publicized report in the British medical journal Lancet in August.

The gene has been seen largely in the deadly E. coli bacteria and on DNA structures that can be easily copied and passed onto other types of bacteria.

The man was hospitalized in April 2009 after returning from India where he had medical treatment. Nakajima declined to say what kind of treatment the man had received in India, citing the man's privacy.

The man had a high fever while staying at a hospital in Tochigi, north of Tokyo. He was discharged in October last year.

The hospital _ Dokkyo Medical University Hospital _ kept a preserved sample of the suspected superbug from the man. The hospital examined the sample after the Lancet report.

The Tochigi hospital notified the Health Ministry about the detection of the NDM-1 gene. It told the ministry that n in-hospital infections were found. Following the confirmation of the discovery _ Japan's first NDM-1 case _ the Health Ministry launched a nationwide survey, asking local health authorities to check on hospitals for evidence of more infections.

Along with India, the new superbug gene has been detected in small numbers in Australia, Canada, the United State the Netherlands, Sweden and the U.K. Researchers say since many Americans and Europeans travel to India and Pakistan for elective procedures like cosmetic surgery, it was likely the superbug gene would spread worldwide.

Antimicrobial resistance _ the ability of microorganisms to escape drugs' efficacy _ is an increasing global health problem that could affect control of diseases such as respiratory infections and dysentery, according to the World Health Organization.

The WHO says NDM-1 requires monitoring and further study. With effective measures, countries have successfully battled multi-drug resistant microorganisms in the past.

It recommends that governments focus their efforts in four areas: surveillance, rational antibiotic use, legislation to stop sales of antibiotics without prescription, and rigorous infection prevention measures such as hand-washing in hospitals.

別紙様式第2-1

No. 30

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
				2010. 10. 4	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		USA TODAY. Available from: http://www.usatoday.com/yourlife/health/medical/2010-09-17-1Asuperbug17_ST_N.htm	米国

研究報告の概要

○強力薬剤耐性菌が米国の35州を襲い、世界へ拡大

抗生物質の機能を一時的に阻害する酵素*Klebsiella pneumoniae* carbapenamase(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35州以上の病院で報告されている。この細菌は重篤な疾患患者を襲い、死亡率は全症例の30～60%に及ぶ。米国疾病管理予防センター(CDC)によると、主にインドで治療を受けた患者において認められたNew Delhi metallo-β-lactamase 1(NDM-1)は米国では稀であり、KPCの方が全国的にはるかに一般的で、現在では米国の半分以上の州で報告されているという。

この細菌に対する唯一の薬にポリミキシンがあるが、腎臓に有毒であるため数年前からほとんど使用されていない。従って予防は極めて重要である。2009年3月にCDCは予防に関する新しいガイドラインを示し、特にカルバペネム耐性菌感染症と診断される患者を治療する医師は、ガウンと手袋を着用して自身を守るとともに、他の患者への感染を防がなくてはならないとしている。

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報告企業の意見	今後の対応
抗生物質の機能を一時的に阻害する酵素*Klebsiella pneumoniae* carbapenamase(KPC)を産生する遺伝子を備えた強力な薬剤耐性菌が米国の35以上の州で報告されているとのことである。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、保存前白血球除去及び初流血除去を導入している。さらに、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ほか、細菌感染が疑われる場合の対応を周知している。細菌やウイルスの検出や不活化する方策について検討している。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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

USA TODAY

Superbug spreading

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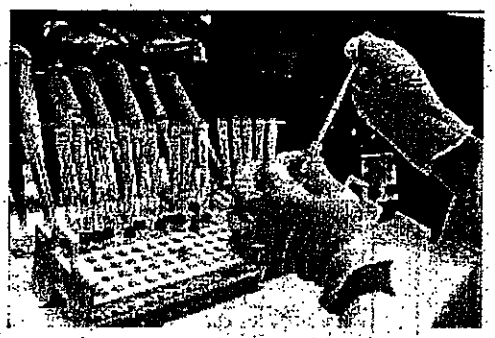
By Steve Sternberg, USA TODAY



By Paul S. Howell for USA TODAY

Medical technician Carla Woodmansee tests nose swabs of new patients for MRSA at UTMB, the University of Texas Medical Center in Nov. 2007. Bacteria that are able to survive every modern antibiotic are cropping up in many U.S. hospitals and are spreading outside the USA, public health officials say.

Bacteria that are able to survive every modern antibiotic are cropping up in many U.S. hospitals and are spreading outside the USA, public health officials say.

The bugs, reported by hospitals in more than 35 states, typically strike the critically ill and are fatal in 30% to 60% of cases. Israeli doctors are battling an outbreak in Tel Aviv that has been traced to a patient from northern New Jersey, says Neil Fishman, director of infection control and epidemiology at the University of Pennsylvania and president of the Society of Healthcare Epidemiologists.

The bacteria are equipped with a gene that enables them to produce an enzyme that disables antibiotics. The enzyme is called Klebsiella pneumoniae carbapenamase, or KPC. It disables carbapenam antibiotics, last-ditch treatments for infections that don't respond to other drugs.

"We've lost our drug of last resort," Fishman says.

Carbapenam-resistant germs are diagnosed mostly in hospital patients and are not spreading in the community. They're far more common nationwide than bacteria carrying a gene called NDM-1 that made headlines this week, Fishman says.

Those NDM-1 bacteria, named for the city of New Delhi, are rare in the USA and have been found mainly in people who obtain medical treatment in India, Arjun Srinivasan of the U.S. Centers for Disease Control and Prevention (CDC) said Thursday.

Although KPCs are most common in New York and New Jersey, Srinivasan says, "they've now been reported in more than half of the states." A decade ago, only 1% of Klebsiella pneumoniae bacteria reported to CDC by hospitals were carbapenam-resistant. Today, resistance has spread to more than 8% of these bacteria. No one knows precisely how many people have KPC infections because cases aren't routinely reported to the CDC.

"We see a ton of the KPC organisms," says Yoko Furuya, medical director of infection control in New York Presbyterian Hospital. "It started in 2002-2003. They just somehow established themselves in nursing homes and hospitals. We always have some patients, five to 10 at a time, in the hospital with this problem."

Doctors say the bacteria are more worrisome than another well-known superbug, methicillin-resistant Staphylococcus aureus (MRSA), because more drugs are available to treat MRSA, Fishman says. "When MRSA started to develop 15 years ago, the industry started producing antibiotics now coming onto the

market," he says. "We're in the same position with KPCs as we were with staph aureus 15 years ago, except that the pharmaceutical industry isn't rushing to produce new drugs."

One of the only drugs that combats these bugs is polymixin, which was all but abandoned years ago because it is so toxic to the kidneys, Fishman says. As a result, he says, prevention is crucial.

In March 2009, the CDC gave hospitals new guidelines for prevention. Among other things, doctors treating any patient diagnosed with carbapenam-resistant infections are advised to wear gowns and gloves to protect themselves and make sure they don't infect other patients.

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	総合機構処理欄
				2010. 10. 4	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		MMWR Vol. 59 No. 36. Available from: http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5936a1.htm?s_cid=mm5936a1_w	米国

研究報告の概要

○臓器移植による*Balamuthia mandrillaris*の伝播ーミシシッピー州、2009年

2009年12月14日、ミシシッピー州の医師により、同じドナーからの腎臓移植レシピエント2名が移植により脳炎を発症した可能性があると米国疾病管理予防センター(CDC)に報告された。CDCはドナーの剖検脳組織からアメーバを発見し、その後、ドナーと2名のレシピエントからの検体で実施した検査により、バラムチア・アメーバ性肉芽腫性脳炎(GAE)の伝播を確認した。これは*Balamuthia mandrillaris*(土壌中に生息する自由生活アメーバ)に起因するまれな疾患である。

腎臓移植レシピエント2名のうち1名(31歳女性)は死亡し、もう1名(27歳男性)は右腕、両脚、視力に後遺症があるが生存している。同じドナーから心臓移植と肝臓移植を受けたレシピエントにはそれぞれ感染の徴候は見られていない。ドナー(4歳の健常男児)はインフルエンザA感染症を発症後、急性散在性脳脊髄炎で死亡したと推定される。

これは臓器移植によるバラムチア感染症の初めての報告である。

使用上の注意記載状況・その他参考事項等

赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-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
臓器移植による初のバラムチア感染症によるアメーバ性肉芽腫脳炎が米国疾病管理予防センターに報告されたとのことである。	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。

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Centers for Disease Control and Prevention
MMWR Morbidity and Mortality Weekly Report
Weekly / Vol. 59 / No. 36 September 17, 2010

Balamuthia mandrillaris Transmitted Through Organ Transplantation — Mississippi, 2009

On December 14, 2009, a physician in Mississippi contacted CDC to report possible transplant-transmitted encephalitis in two kidney transplant recipients who shared the same organ donor. Histopathologic testing of donor autopsy brain tissue at CDC showed amebae, and subsequent testing of specimens from the donor and the two kidney recipients confirmed transmission by transplantation of *Balamuthia* granulomatous amebic encephalitis (GAE), a rare disease caused by *Balamuthia mandrillaris*, a free-living ameba found in soil (*1*). One kidney recipient, a woman aged 31 years, died; the other recipient, a man aged 27 years, survived with neurologic sequelae. Recipients of the heart and liver from the same donor received preemptive therapy and have shown no signs of infection. The donor, a previously healthy boy aged 4 years, was presumed to have died from acute disseminated encephalomyelitis (ADEM), an autoimmune neurologic disease, after infection with influenza A. An investigation was conducted by the state health departments in Mississippi, Kentucky, Florida, and Alabama and CDC to characterize the cases, elucidate possible exposures in the donor, and develop recommendations for early detection and prevention. This is the first reported transmission of *Balamuthia* by organ transplantation. Clinicians should be aware of *Balamuthia* infection as a potentially fatal cause of encephalitis. Organ procurement organizations (OPOs) and transplant centers should be aware of the potential for *Balamuthia* infection in donors with encephalitis of uncertain etiology, and OPOs should communicate this elevated risk for infection to transplant centers so they can make an informed risk assessment in the decision to accept an organ.

Organ Donor

The organ donor, a boy aged 4 years from Kentucky, was living with relatives in Mississippi in October 2009, when he developed a transient febrile illness. He was diagnosed with influenza A infection by rapid influenza test on October 25 and prescribed antivirals; his symptoms resolved without hospitalization. On November 3, the boy had sudden onset of headache and seizures and was hospitalized (Table 1). Cerebrospinal fluid (CSF) demonstrated lymphocytic pleocytosis (170 white blood cells/mm^3) and normal protein (29 mg/dL); magnetic resonance imaging (MRI) of the brain showed numerous small enhancing lesions and edema (Table 2). An extensive search for viral, bacterial, and fungal etiologies of encephalitis was unrevealing. His clinical presentation, CSF findings, and MRI were thought to be most consistent with a diagnosis of ADEM, an immune-mediated encephalitis that can follow influenza or other infections. He was treated symptomatically and discharged on November 6.

The boy was readmitted on November 10 with recurrent seizures. MRI of the brain demonstrated progression of several of the enhancing lesions; CSF again demonstrated lymphocytic pleocytosis (150 cells/mm^3) and normal protein (44 mg/dL) (Table 2). He was treated for presumed worsening ADEM with intravenous corticosteroids and immunoglobulin. He developed subarachnoid hemorrhage and brain stem herniation on November 18 and was pronounced brain dead the next day. His heart, liver, and kidneys were transplanted into four recipients at three different transplant centers on November 20. On December 16, histopathologic examination of the donor's

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brain tissue at CDC revealed the presence of abundant amebae morphologically suggestive of *Balamuthia* (Figure); empiric treatment for both kidney recipients was initiated later that day, in consultation with CDC. On December 17, immunohistochemical and indirect immunofluorescent stains (Figure) revealed antigens of free-living amebae in the donor's brain tissue; polymerase chain reaction (PCR) results confirmed *Balamuthia* infection.

Kidney Recipient A

Kidney recipient A, a woman aged 31 years, underwent transplantation for end-stage renal disease resulting from hypertension and diabetes. On December 10, post-transplant day (PTD) 20, she reported onset of right leg twitching and neck spasms, numbness, headache, nausea, and seeing flashing lights (Table 1). She was evaluated in an emergency department, where she was treated with benzodiazepines and discharged with muscle relaxants; no neuroimaging or lumbar puncture was performed. On December 12, she was found unresponsive at home and taken back to the emergency department, where she had a generalized seizure and was admitted; the next day, she was transferred to the intensive-care unit. MRI of the brain demonstrated numerous ring-enhancing lesions. CSF initially showed a normal white blood cell count (3 cells/mm^3) and elevated protein (75 mg/dL); however, another specimen collected on December 15 revealed a neutrophilic pleocytosis (507 cells/mm^3) and increased protein (142 mg/dL) (Table 2). On December 16, she underwent brain biopsy. On December 18, histopathologic examination of the brain tissue at CDC revealed amebae; immunohistochemical stains detected antigens of free-living amebae, and PCR confirmed *Balamuthia* infection. She was treated with pentamidine, sulfadiazine, flucytosine, fluconazole, and azithromycin. Miltefosine, an antileishmanial and antineoplastic agent, was added on December 25 under an emergency investigational new drug (IND) protocol. Despite several weeks of intensive care, she deteriorated neurologically and died on February 3 (PTD 75).

Kidney Recipient B

Kidney recipient B, a man aged 27 years, underwent transplantation for end-stage renal disease resulting from focal segmental glomerulosclerosis. On December 10 (PTD 20), he had sudden onset

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TABLE 1. Timeline of events involving transmission of *Balamuthia* infection from an organ donor to two kidney recipients — Mississippi, 2009–2010

Date	Donor	Kidney recipient A	Kidney recipient B
2009			
November 3	Hospitalized with headaches and seizures.		
November 5	Initial brain MRI performed.		
November 6	Discharged from hospital.		
November 10	Hospitalized after recurrence of seizures.		
November 18	Developed subarachnoid hemorrhage and brain stem herniation.		
November 19	Pronounced brain dead.		
November 20	Heart, liver, and kidneys transplanted into four recipients.	Received kidney from donor.	Received kidney from donor.
December 10		Onset of right leg twitching and neck spasms, numbness, headache, nausea, and seeing flashing lights.	Onset of severe headache and vomiting.
December 11			Onset of altered mental status and seizures. Hospitalized.
December 12		Found unresponsive at home. Hospitalized.	
December 13		Admitted to intensive-care unit.	Admitted to intensive-care unit. Initial brain MRI performed.
December 15		Initial brain MRI performed.	
December 16	Histopathologic examination of brain tissue at CDC revealed amebae suggestive of *Balamuthia*.	Underwent brain biopsy. Started on multiple drug regimen.	Started on multiple drug regimen.
December 17	*Balamuthia* infection confirmed by PCR.	Amebae seen on brain histopathology at CDC.	
December 18		*Balamuthia* infection confirmed by PCR of brain tissue at CDC.	
2010			
January 5			*Balamuthia* infection confirmed by PCR on CSF specimen drawn December 29.
February 2			*Balamuthia* infection cultured from CSF specimen drawn December 29.
February 3		Died after 7 weeks of intensive care.	
April 28			Discharged to a rehabilitation facility.
June 11			Discharged home with neurologic sequelae.

Abbreviations: MRI = magnetic resonance imaging; PCR = polymerase chain reaction; CSF = cerebrospinal fluid.

of severe headache and vomiting and was examined at a local emergency department early the next morning, where he was diagnosed with sinusitis and discharged on amoxicillin-clavulanic acid (Table 1). Later that day, he developed altered mental status and seizures and was admitted to a regional hospital. A lumbar puncture was performed; CSF demonstrated 1 white blood cell/mm^3 and slightly elevated protein (69 mg/dL) (Table 2). On December 13, he was transferred to the intensive-care unit at the same hospital as kidney recipient A. CSF that day revealed mild pleocytosis (19 cells/mm^3) and slightly increased protein (74 mg/dL). MRI of the brain showed numerous ring-enhancing lesions. The man was treated with the same combination of drugs as kidney recipient A, including miltefosine obtained under IND. *Balamuthia* infection was confirmed by PCR and culture on a CSF specimen drawn December 29. After 2 months in a coma, the man had a slow but significant recovery of cognitive and motor function and was discharged to a rehabilitation facility on April 28 (PTD 159). He was discharged home June 11. His neurologic sequelae included residual right arm paralysis, bilateral leg weakness, and intermittent vision loss; however, he performed most activities of daily living independently.

Heart Recipient

The heart recipient, a boy aged 2 years, underwent transplantation for restrictive cardiomyopathy. When the

TABLE 2. Demographic, clinical, and laboratory features of cases involving transmission of *Balamuthia* infection from an organ donor to two kidney recipients — Mississippi, 2009–2010

Patient	Age/Sex	Race/ Ethnicity	Time from transplant to symptom onset	Initial clinical symptoms	Initial lumbar puncture (LP) results (2nd LP results) WBC*	Protein†	Glucose§	Neuroimaging results	Mode of initial *Balamuthia* GAE diagnosis	Preliminary diagnosis	Outcome
Donor	4 yrs/male	White, non-*Hispanic*	N/A	Personality changes, loss of appetite, muscle twitching, *headache, seizure*	170 (150)	29 (44)	49 (46)	Multiple focal enhancing *lesions*	Autopsy	ADEM	Death
Kidney recipient A	31 yrs/female	Black, non-Hispanic	20 days	Paresthesias, muscle spasms, headache, nausea, altered mental status, seizure	3 (507)	75 (142)	114 (67)	Multiple large ring-enhancing lesions	PCR of brain biopsy	Muscle spasms	Death
Kidney recipient B	27 yrs/male	Black, non-Hispanic	20 days	Headache, nausea, altered mental status, seizure	1 (19)	69 (74)	77 (62)	Ring-enhancing lesions	PCR and culture of CSF	Sinusitis	Survived, but with neurologic sequelae¶

Abbreviations: GAE = granulomatous amebic encephalitis; ADEM = acute disseminated encephalomyelitis; PCR = polymerase chain reaction; CSF = cerebrospinal fluid.
* White blood cells per mm^3; normal range: 0–5 (aged >12 yrs), 0–20 (aged 1–4 yrs).
† mg/dL; normal range: 12–60.
§ mg/dL; normal range: 40–70.
¶ Including intermittent hemianopsia, bilateral leg weakness, and right arm paralysis.

kidney recipients were diagnosed with *Balamuthia* GAE, the boy was asymptomatic. On December 17 (PTD 27), he was hospitalized for evaluation. MRI of the brain was normal, and testing of CSF, serum, and endomyocardial tissue at CDC showed no evidence of *Balamuthia* infection. The boy was treated for presumed *Balamuthia* exposure with a 6-week course of intravenous pentamidine, azithromycin, and fluconazole, followed by 5 weeks of oral azithromycin. He remains well.

Liver Recipient

The liver recipient, a boy aged 7 years, underwent transplantation for end-stage liver disease resulting from alpha-1-antitrypsin deficiency. The boy was asymptomatic when the kidney recipients were diagnosed with *Balamuthia* GAE, and he was hospitalized for evaluation on December 17. MRI of the boy's brain was normal, and testing of CSF, serum, and liver tissue at CDC showed no evidence of *Balamuthia* infection. He was treated for presumed *Balamuthia* exposure with a 1-month course of intravenous pentamidine, fluconazole, azithromycin, and sulfadiazine. He remains well.

Public Health Investigation

Interviews with the donor's family revealed that he had lived in Kentucky, Florida, and Mississippi during the 2 years before his death. He frequently played outdoors and had soil exposure in all of these locations. He occasionally played in a wading pool; the water supply for drinking and recreation in Florida was untreated well water. No environmental sampling was performed because *Balamuthia* is thought to be ubiquitous in the environment.

Approximately 4 months before his first seizure, the boy had become more irritable and emotionally labile. His family also noted regression of toilet training and an infrequent, sporadic tremor of the right hand that began at about the same time. He had no history of immunocompromising conditions. No medical evaluation of family members was conducted.

Reported by

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Editorial Note

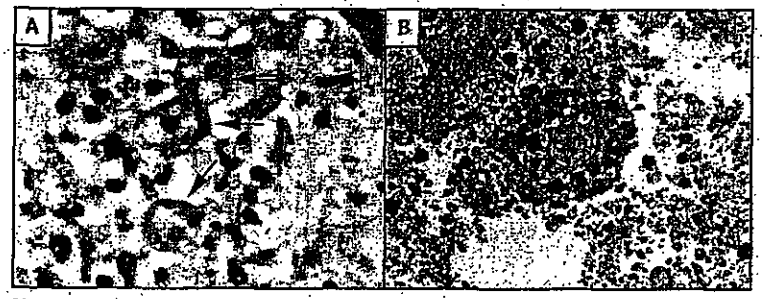
This report is the first to describe transmission of *Balamuthia* through organ transplantation. However, a second cluster of patients with transplant-transmitted *Balamuthia* was confirmed at CDC on August 27, 2010 (*2*). *Balamuthia* infection is extremely rare, with fewer than 200 human cases recognized worldwide since *Balamuthia* was found to be a human pathogen in 1990 (*3,4*). The true magnitude of disease caused by *Balamuthia* is unknown because *Balamuthia* GAE often is misdiagnosed as other neurologic diseases (*1,3*). Once infection progresses to encephalitis, it is almost always fatal. Infection occurs in both immunocompromised and otherwise healthy persons, and often in children, although cases have occurred in patients across the age spectrum (*5*). Because of the rarity of *Balamuthia* GAE, risk factors are poorly defined, but might include exposure to soil or stagnant water, young age, and Hispanic ethnicity (*3*).

Balamuthia has been isolated from soil and dust and is thought to be present worldwide (*6*). Routes of infection might include exposure of mucous membranes or nonintact skin to cysts or trophozoites in soil. *Balamuthia* has not been isolated from water, but water also might serve as a vehicle for infection (*1*). Cutaneous lesions have preceded *Balamuthia* GAE in some cases, primarily those reported in South America (*7*). These lesions often are on the central face, suggesting nasal exposure; but they also have been reported on the extremities. Extension to the brain might occur through hematogenous spread or by direct extension through the nasal cavity or sinuses (*1*). Why some patients develop cutaneous lesions before onset of neurologic disease and others do not is unknown. In a series of 10 *Balamuthia* cases in California, common signs and symptoms of *Balamuthia* GAE were headache, altered mental status, and cranial nerve abnormalities (*3*). Although the incubation period for *Balamuthia* GAE has been postulated as ranging from weeks to 2 years, the two kidney recipients in this report had onset of symptoms only 20 days after transplantation.

Successful treatment of *Balamuthia* GAE has been reported in some, but not all, patients administered a combination of flucytosine, pentamidine, sulfadiazine, fluconazole or amphotericin B, azithromycin or clarithromycin, and miltefosine (*3,8*). However, optimal therapy has not been determined. Optimal preemptive therapy for asymptomatic recipients after transplant of an infected organ also is unknown. Miltefosine is active against *Balamuthia* in vitro and was recently used with success in combination therapy for *Balamuthia* GAE in Peru (*9*). Miltefosine is not marketed in the United States but can be available through single patient IND.*

Balamuthia is one of several agents of severe or fatal encephalitis (e.g., West Nile virus, lymphocytic choriomeningitis virus, and rabies virus) that have been transmitted through organ transplantation in recent years (*10*). Organ donors are screened to identify infectious risks in accordance with policies set by the Organ Procurement and Transplantation Network,† which is overseen by the United Network for Organ Sharing through a contract with the Health Resources and Services Administration. However, the number of pathogens screened is limited and creating standards that eliminate all risk for infectious disease transmission is not feasible. Therefore, physicians and organ procurement organizations should be aware of the possibility of transmitting *Balamuthia* and other potentially fatal infections from donors with encephalitis of uncertain etiology, even after testing for usual agents of encephalitis has shown negative results (*10*). *Balamuthia* infection should be considered in patients who might have an infectious encephalitis,

FIGURE. Organ donor brain tissue revealing amebae suggestive of *Balamuthia* (indicated by arrows) (A), and immunohistochemical staining showing antigens (red) of free-living amebae (B)*



Photomicrographs/CDC

* Original magnifications: 158x (A), 100x (B).

* For information regarding a single patient IND for miltefosine, contact the Food and Drug Administration's Division of Special Pathogen and Transplant Products at 301-796-1600 (1-888-INFO-FDA after hours).

† Additional information available at http://optn.transplant.hrsa.gov/policiesandbylaws/policies.asp.

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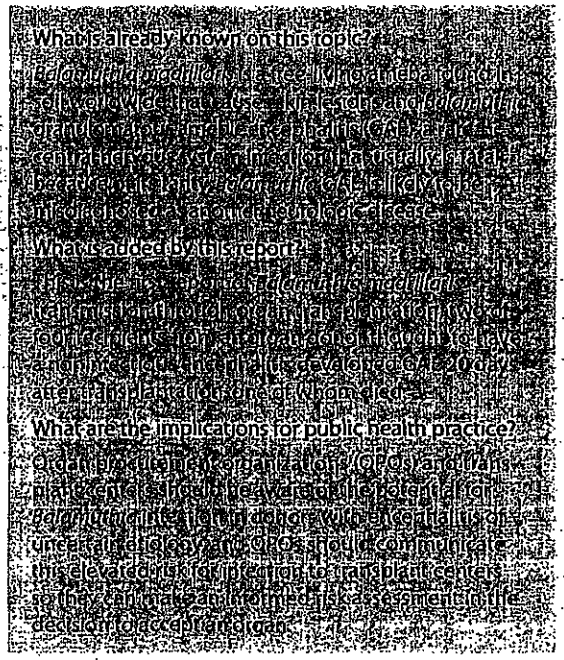

particularly those with elevated CSF protein, CSF pleocytosis (white blood cells >5/mm^3), and enhancing lesions on MRI (*3*).

Clinicians should be aware of *Balamuthia* as a cause of skin lesions and encephalitis and should report all suspected cases of transplant-transmitted infection to public health departments and organ procurement organizations to enable prompt evaluation and treatment of all recipients from an infected donor. OPOs and transplant centers should be aware of the potential for *Balamuthia* infection in donors with encephalitis of uncertain etiology, and OPOs should communicate this elevated risk for infection to transplant centers so they can make an informed risk assessment in the decision to accept an organ.[§]

[§]Additional information available at http://www.cdc.gov/balamuthia.

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別紙様式第 2-1
番号 10

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 2010 年 10 月 14 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②③ポリエチレングリコール処理抗破傷風人免疫グロブリン ④⑤乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	Journal of Pathology 2010; 10(1002): 2767-2767	公表国 イギリス
販売名 （企業名）	①テタノブリン IH 静注 250 単位　（ベネシス） ②テタノブリン IH 静注 1500 単位　（ベネシス） ③テタノブリン-IH　（ベネシス） ④テタノブリン筋注用 250 単位　（ベネシス） ⑤テタノブリン　（ベネシス）			

研究報告の概要

英国で集められた扁桃腺標本のリンパ網内プリオン蛋白の大規模な免疫組織化学試験についての報告である。
BSE の結果として、2010/7/5 現在までに英国では 173 の vCJD 症例がある。無症候性感染の数および最終的な患者数は不確かである。この問題を解決する計画で、63,007 の扁桃腺組織標本で過去に PrPres 関連疾患の存在について EIA 法により調査され、陰性であった。この結果の信頼性を確認するために、最もリスクのあるコホート（1961-1985 年生まれ）について免疫組織化学的に試験された。
扁桃腺 9160 検体の中の 1 検体が陽性であり、英国人口 100 万人あたり 109 人のプリオン蛋白関連疾患の保有率であった。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
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
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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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ORIGINAL PAPER

Large-scale immunohistochemical examination for lymphoreticular prion protein in tonsil specimens collected in Britain

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Abstract

There have been 173 cases of variant Creutzfeldt–Jakob disease (vCJD) in the UK, as of 5 July 2010, as a result of the bovine spongiform encephalopathy epidemic. The number of individuals subclinically infected with vCJD, and thus the eventual number of cases, remains, however, uncertain. In an attempt to address this problem, 63 007 tonsil tissue specimens were previously tested by enzyme immunoassay (EIA) for the presence of disease-related prion protein (PrP^{res}) and found to be negative. To confirm the reliability of this result, all those in the birth cohort most at risk (1961–1985) and a few others, including controls, have now been tested by immunohistochemistry (IHC). Histological slides were prepared from 10 075 anonymized formalin-fixed, paraffin-embedded tissues and examined for PrP^{res} with two anti-prion protein antibodies, ICMS35 and KG9. One specimen showed a single strongly positive follicle with both antibodies, on two slides from adjacent sections. As this specimen was negative when it was further investigated by EIA, IHC, and immunoblotting, it is unclear whether the patient from whom the tonsil came will go on to develop vCJD. If, however, this is the case, then a finding of 1 out of 9160 gives a prevalence of disease-related prion protein in the British population of 109 per million, with a 95% confidence interval (CI) of 3–608 per million, which is not statistically different (exact $p = 0.63$) from population prevalence estimates based on finding three positives out of 10 278 in a previous IHC study of appendix tissue. If this is not the case, a finding of 0 out of 9160 gives a prevalence of 0–403 per million (95% CI) for the 1961–1985 cohort, which is also not different (exact $p = 0.25$) from previous population prevalence estimates. Therefore, the results of this work could be summarized as finding, by IHC, no or one vCJD-positive individual.

Keywords: variant Creutzfeldt–Jakob disease; bovine spongiform encephalopathy; vCJD prevalence; PrP

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No conflicts of interest were declared.

Introduction

Variant Creutzfeldt–Jakob disease (vCJD) is understood to have arisen from bovine spongiform encephalopathy (BSE) [1–3]. There was widespread population exposure in the UK and some other countries to BSE and as of 5 July 2010, at least 220 people have developed clinical vCJD worldwide (173 in the UK) [4]. The number of currently subclinically infected individuals, and thus the eventual number of cases, remains uncertain [4–6]. This represents an ongoing public health concern with the risk of iatrogenic transmission through blood and surgical instruments [7,8], since prions resist most conventional decontamination procedures [9]. Four instances of vCJD infection resulting from blood transfusion have been reported, establishing the existence of an infective asymptomatic stage [10–14]. There has been a report of autopsy finding of abnormal prion protein (PrP^{res}) in the spleen of a person with haemophilia [15]. Iatrogenic transmission of sporadic CJD has also been reported to occur through neurosurgical instruments, and experimental studies have shown stainless steel-bound prions to transmit disease with remarkable efficiency when implanted into mice [16–18]. These factors, together with the unknown maximum length of the asymptomatic incubation period and the influence of the host's genotype [19–21], all contribute to the uncertainty about the underlying prevalence of vCJD.

Preclinical colonization of the lymphoreticular system in vCJD is lent support by the detection of PrP^{res} in an appendix removed 8 months before onset of overt

neurological symptoms in a patient whose diagnosis was confirmed at autopsy [22,23]. The finding of PrP^{res} in the spleen removed at autopsy from a person with haemophilia is consistent with lymphatic spread of disease [15], as is the report of PrP^{res}, but not disease, in spleen and lymph tissue at post-mortem from a recipient of red blood cells donated by a vCJD case approximately 18 months before onset of clinical symptoms [12]. Moreover, tonsil biopsies are successfully used for the diagnosis of vCJD, showing 100% sensitivity and specificity [24,25]. These collective data indicate that large-scale screening of surgical tonsillectomy tissues for PrP^{CJD} could provide early warning of a high level of subclinical prevalence of vCJD prion in the general population [6,22,24,26,27].

Three previous studies analysed appendix and tonsil specimens for the presence of PrP^{res} [6,26,27]. In the first study, 11 247 archived fixed appendix specimens, and 1427 tonsil specimens, were screened by immunohistochemistry (IHC), revealing PrP^{res} deposition in three appendix specimens, all from the 1961–1985 birth cohort [26]. The prevalence of detectable PrP^{res} in Britain was therefore calculated to be 292 (95% confidence interval 60–853) per million [26]. In a second study, 2000 tonsils were screened by both immunoblotting (IB) and IHC, showing no positive cases [27]. A third study examined 63 007 tonsil specimens from a national anonymous tissue archive and screened for the presence of the PrP^{res} by the use of two enzyme immunoassays (EIAs) based on different analytical principles [6]. No samples contained detectable levels, allowing a prevalence estimate of 0 per million (upper 95% confidence limit of 113 per million). These results suggest that the prevalence of subclinical vCJD infection in Britain may be lower than, but still consistent with, that given by the survey of appendix tissue [26], with an upper limit in tonsil tissue of 289 per million in the 1961–1985 birth cohort [6]. These two surveys may not, however, be directly comparable, particularly because the study of Hilton *et al* [26] screened all of the samples by IHC, whereas the study of Clewley *et al* [6] used EIA as the screening method and only employed IHC as a confirmatory method on a limited subset of the 63 007 tonsils.

The aim of the present study was to investigate further, by IHC, the prevalence of subclinical vCJD in Britain in anonymized samples derived from patients in the 1961–1995 birth cohort of the 63 007 tonsils collected by Clewley *et al* [6].

Materials and methods

Tonsil archive

The tonsils used in this study came from an opportunistic sample of 63 007 tonsils removed for clinical reasons at 131 hospitals across England and Scotland representing 11 Strategic Health Authorities (South West, South Central, South East Coast, East of England, London, East Midlands, West Midlands, Yorkshire and the Humber, North East, North West, and Scotland) [6]. All available tonsils from patients in the 1961–1985 birth cohort were selected for testing by IHC, as well as others chosen for control and technical reasons. The study received ethical approval from the Trent Multi-Centre Research Ethics Committee (MREC/03/4/073) [6].

Sectioning and conventional staining

Sections were prepared from tissue blocks at a nominal thickness of 5 μm at two levels using a standard rotary microtome (LEICA RM 2135, LEICA, Milton Keynes, UK). One section per block was stained with H&E for morphological assessment using standard staining procedures, on a LEICA Autostainer ST5020.

Immunohistochemical staining

For immunohistochemical analysis, sections of formalin-fixed tonsils were dewaxed and rehydrated, immersed into 98% formic acid for 5 min, and then washed in PBS. Thereafter, the slides were loaded onto a BondMax automated immunostaining instrument (LEICA). All antigen retrieval, staining, washing, and haematoxylin counterstaining steps were carried out on this instrument. The antigen retrieval was performed using Bond™ Epitope Retrieval solution 1 and Bond™ Enzyme (LEICA Microsystems, Milton Keynes, UK). Endogenous peroxidase was neutralized and the sections were incubated with either anti-PrP monoclonal antibody ICSM35 (D-Gen, London, UK; dilution 1:1500) or anti-PrP monoclonal antibody KG9 (Institute for Animal Health, TSE Resource Centre, UK; dilution 1:3000). ICSM35 recognizes the region encompassing residues 93 and 102 of human PrP, and KG9 recognizes residues 140–180 of human PrP. The sections were then incubated with the secondary antibody for signal amplification and detection (Vision Biosystems Bond Polymer Detection System, visualized with diaminobenzidine and Bond DAB Enhancer). After counterstaining with haematoxylin, the sections were dehydrated in ascending concentrations of alcohols and xylene and coverslipped with a LEICA ST5020 automated coverslipper.

Autopsy brain tissues from confirmed cases of CJD were used as a positive control for each machine cycle, while omission of the primary antibody on a CJD brain section served as a negative control. Blinded positive control sheep scrapie tonsil tissue cases were randomly included among the human tonsil tissue blocks as an internal quality control, to test the overall sensitivity of the IHC screening.

Each section was labelled with the unique identifier number, the date of the run, and a unique identifier that can be linked to all data related to the machine cycle, reagent and batch number, incubation times, and other parameters.

Microscopic examination

A first quality control was performed by evaluating the quality of the staining in the controls and in the tonsil specimens. We examined and scored entire sections of every anonymous tonsil specimen, at one or more levels, after inspection of a minimum of 15 lymphoid follicles or a minimum of 20 mm^2 tonsil area.

Additional testing

EIA screening, immunoblotting (IB), and codon 129 genotyping were carried out as previously described [6]. For further investigatory IHC, the existing and new wax blocks were sent to two independent laboratories (CJD Surveillance Unit, Edinburgh and Derriford Hospital, Plymouth, UK), where they were tested as previously described [6,26]. Confirmatory enhanced chemiluminescent IB was carried out by the MRC Prion Unit [25,27] and the National CJD Surveillance Unit [11,28].

Statistical methods

95% confidence intervals for prevalence estimates were calculated using the exact binomial method, and comparisons of prevalence between surveys were made using Fisher's exact test.

Results

We examined a total of 24360 slides by IHC, of which 17% were repeated because of failure of IHC or insufficient control staining (11%), enzymic overdigestion, irregular distribution of DAB, poor sectioning, etc (5%). One per cent of the slides were repeated for further investigation, due to the presence of suspicious staining. From 10075 tonsil specimens, 4% were rejected due to the absence or too small an amount of lymphoid tissue, or because of poor tissue quality. Of the 9675 tonsil specimens accepted for the study, 94% had more than 30 lymphatic follicles, 5.5% contained 15–30 follicles, and only 0.5% had a minimum diagnosable area. We screened about 20% of anonymous tonsil specimens more than once. We found three samples with positive labelling requiring further examination to confirm or exclude specific labelling for PrP^{res}.

The first specimen, 38660 (Figures 1A–1D, and Table 1), when stained with ICSM35 (Figure 1A) and KG9 (Figure 1B), showed intense but diffuse staining in an identical follicle. However, while immunoreactivity was not seen elsewhere in the section stained with KG9, we could detect non-specific staining when the specimen was stained with ICSM35. Therefore, new sections were stained with ICSM35 or KG9 (Figures 1C and 1D), showing no positive staining in the follicle that was positive before. These slides were independently examined, with the conclusion that although the pattern of follicular dendritic cell (FDC) staining in this case does not have the coarse granularity seen in positive tonsils from symptomatic cases, it should be classed as positive on the basis of the one strongly positive follicle with both KG9 and ICSM35 antibodies (J Ironside and D Hilton, personal communication).

A second specimen, 18824 (Figures 1E–1H, and Table 1), showed strong but diffuse, rather than specific FDC staining. This tonsil showed three adjacent follicles positive in one margin of the specimen when stained with ICSM35 (Figure 1E), together with non-specific staining elsewhere in the tonsil. The same follicles were positive in the KG9 staining in an adjacent section (Figure 1F). Because the staining was suspicious but not typical, the staining was repeated on an adjacent section on a different instrument using antibody ICSM35 and established protocols (dilution 1 : 3000). Still there was speckled positive labelling of unclear significance in the three follicles that were positive before and no staining elsewhere (Figure 1G). On the basis of this result, new sections were prepared and stained, showing perinuclear positivity in the same (immediately adjacent) follicles (Figure 1H). These slides were independently examined, with the conclusion that the staining seen was 'background' (J Ironside and D Hilton, personal communication).

A third specimen, 40751 (Figures 1I–1N, and Table 1), was stained with ICSM35 (Figure 1I) and KG9 (Figure 1L). Although no immunoreactivity was found in both slides, ICSM35 staining was repeated because of poor staining quality. Therefore, a new section was stained using ICSM35. Immunoreactivity was then detected in one lymphoid follicle, showing a fine granular pattern suggesting FDC positivity (Figures 1J and 1M). We therefore repeated the staining again on new sections, again with ICSM35 (Figure 1K) and KG9 (Figure 1N), respectively. This (second) repeat showed no immunoreactivity. These slides were independently examined, with the conclusion that the staining seen was 'probable background' (J Ironside and D Hilton, personal communication).

These three samples were further investigated by EIA, IB, IHC, and codon 129 genotyping (Table 2). They had all given negative results in the initial EIA screening, and this was confirmed on repeat testing after the IHC findings were reported. IB by both the Prionics (G Mallinson, personal communication) and the Bio-Rad methods was negative for all three samples. In addition, multiple tissue homogenates were referred as blinded samples for enhanced chemiluminescent IB [11,25,27,28] and were reported as negative (J Wadsworth and M Head, personal communication). Additional tissue blocks in wax were made from each of these three samples and they were independently examined and reported as negative (J Ironside and D Hilton, personal communication).

Tonsils 38660 and 40751 were both MV heterozygotes, whereas tonsil 18824 originated from a patient homozygous for valine at codon 129 of the *PRNP* gene.

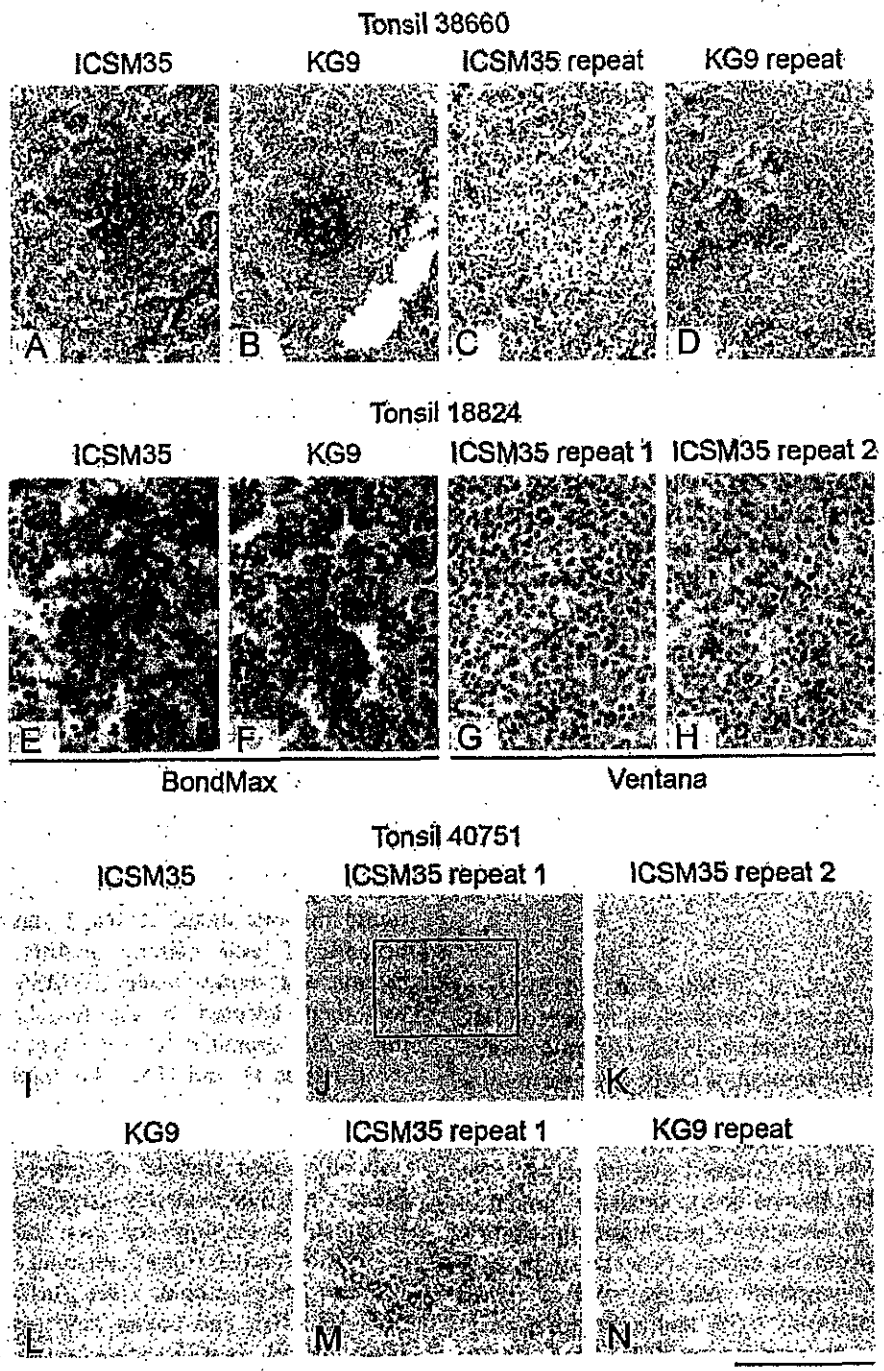

Figure 1. Tonsil specimens with positive immunolabelling. (A–D) Immunoreactivity in tonsil specimen 38660 stained with ICSM35 (A), KG9 (B), ICSM35—new sections (C), and KG9—new sections (D). (E–H) Non-specific immunoreactivity in tonsil specimen 18824 stained with anti-PrP antibody ICSM35 (E), anti-PrP antibody KG9 (F), ICSM35—first repeat (G), and ICSM35—second repeat (H). (I–N) Immunoreactivity in tonsil specimen 40751 stained with ICSM35 (I), KG9 (L), ICSM35—first repeat (J, M), ICSM35—second repeat (K), and KG9—repeat (N). Scale bar: 320 μm (all images).

All three samples were from patients in the 1981–1985 birth cohort.

Three internal quality control sheep scrapie tonsil tissues were successfully detected, although only when stained with anti-PrP monoclonal antibody ICSM35. None of these tonsils showed immunoreactivity when stained with anti-PrP antibody KG9 (Figures 2G–2I and Table 1). The sheep tonsils showed a different morphology to that of human tonsils. The first positive control sample showed immunoreactivity with ICSM35 in most of the follicles, with a fine granular pattern in a distribution compatible with FDCs, including also some coarse granular aggregates (Figure 2A). The presence of granular staining outside the follicles was also detected. The second positive control sample showed a mixture of fine granular staining in cells with the morphology of FDCs, including coarse granular aggregates, and accumulation within the cytoplasm

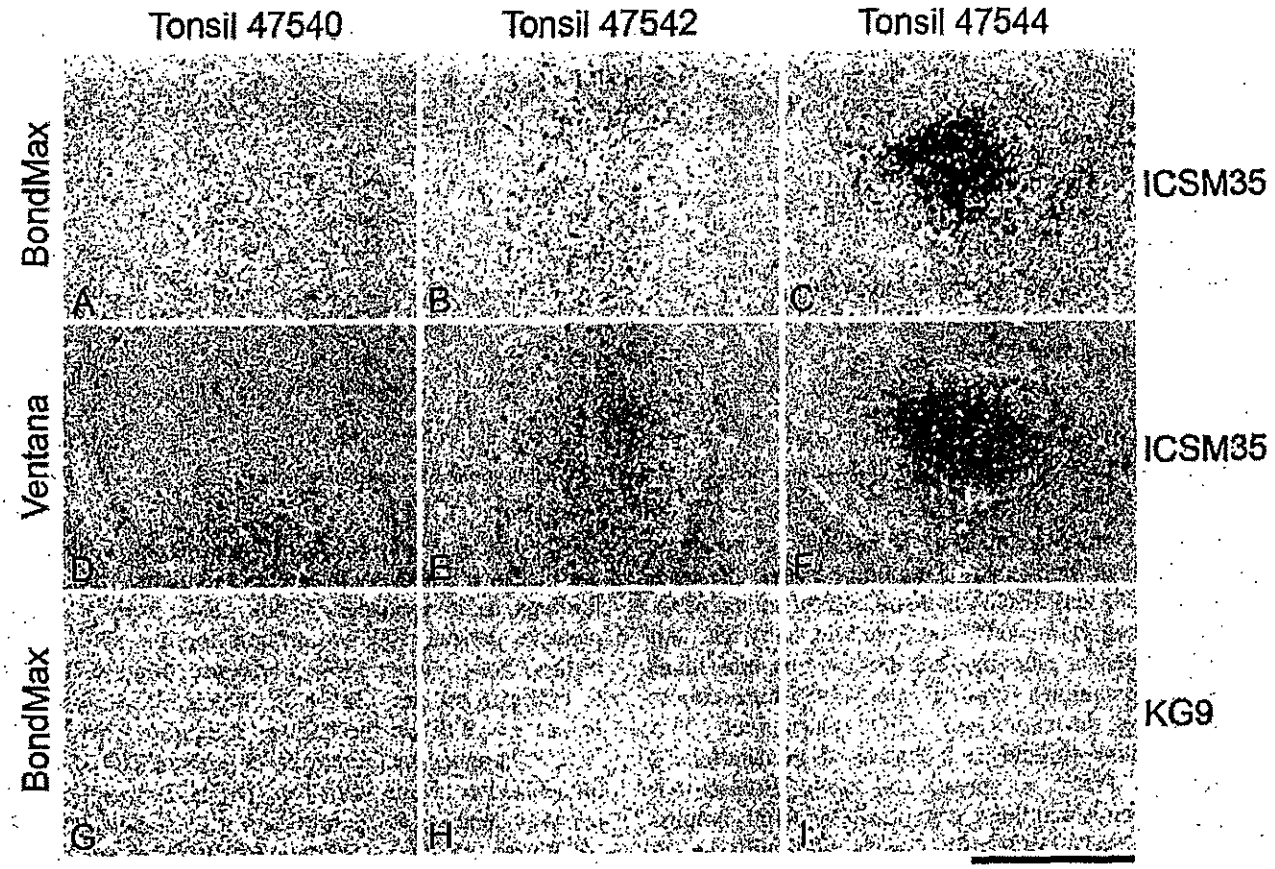

Figure 2. Immunoreactivity in the three blinded positive control sheep scrapie tonsil tissue specimens. Specimen 47540 stained with ICSM35 (A, D) or KG9 (G). Specimen 47542 stained with ICSM35 (B, E) or KG9 (H). Specimen 47544 stained with ICSM35 (C, F) or KG9 (I). Scale bar: 180 μm.

Table 1. Human tonsil tissues reactive by IHC in the 1981–1985 birth cohort

ID No.	Figure	ICSM35	KG9	Interpretation of IHC	IB	EIA* result	*PRNP* codon 129
38660	1A–1D	Initial reactivity	Initial reactivity	One strongly positive follicle	Neg*	Neg†	MV
40751	1E–1H	Initial reactivity	Initial reactivity	Probable background staining	Neg*	Neg†	MV
18824	1I–1N	Initial reactivity	Initial reactivity	Background staining	Neg*	Neg†	W

*By four independent methods: Bio-Rad, Prionics, and two 'in-house' methods. †By two independent methods: Bio-Rad and Microsens.

of macrophages in most of the lymphoid follicles when stained for ICSM35 (Figure 2B). The third positive control sample showed strong staining in a large area within most of the lymphoid follicles when stained with ICSM35, with a distribution suggesting that it was within FDCs (Figure 2C). These three control specimens were then additionally stained on a different instrument (Ventana Medical Systems) using ICSM35 and established protocols (primary antibody dilution 1:3000), confirming the positive result described previously (Figures 2D, 2E, and 2F, respectively).

Discussion

Of the 9675 samples for which an IHC result was obtained, 9160 were in the 1961–1985 birth cohort. The remainder of the samples were selected for IHC because they showed some reactivity in the original serological screening of the 63 007 tonsils by EIA with Bio-Rad and Microsens kits [6]. In addition, there were three positive controls (sheep scrapie) among the 9675 samples submitted for IHC. Three samples (18 864, 38 660, and 40 751) gave IHC results that needed to be investigated more fully. Two of these IHC results were concluded to be background staining by three experts, while for the third it was concluded that there was one strongly positive follicle with both KG9 and ICSM35 antibodies. This could not be confirmed by analysis of slides made from further tissue samples embedded in wax, neither could it be confirmed by IB. This result raises the question of the significance and interpretation of a single positive follicle among the thousands from several sections that were examined, particularly in the light of the failure of IB to confirm the presence of PrP^{CJD} in the tissue. Further investigation of tissue from this specimen by bioassay or protein misfolding cyclic amplification (PMCA) was considered not to be

Table 2. Prevalence of disease-associated prion protein (PrP^{CJD}) in Britain: positive/total and rate per million with 95% confidence intervals

Survey		Birth cohort: 1961–1985	Birth cohort: All tested
2004–September 2008 national tonsil survey by IHC		1/9160*	1/9672
Interpretation: one positive patient		109 (3–608)†	103 (3–576)
2004–September 2008 national tonsil survey by IHC		0/9160	0/9672
Interpretation: zero positive patients		0 (0–403)	0 (0–378)
2004–September 2008 national tonsil survey by EIA		0/12 753	0/63 007
		0 (0–289)	0 (0–59)
1995–1999 national tissue survey by IHC	Appendices	3/10 278	3/11 247
		292 (60–853)	267 (55–779)
	Tonsils	0/694	0/1427
		NA	0 (0–2582)

*Positive/total. †Rate per million with 95% CIs. NA = not applicable, as the 95% CI is calculated only when the denominator exceeds 1000.

worthwhile because bioassay is unlikely to be more sensitive than enhanced chemiluminescent IB tests [11,25,27,28] and PMCA is insufficiently robust [29].

Our finding of one PrP^{res}-positive follicle by IHC can be interpreted as showing that there is one individual in the 9160 samples from the 1961–1985 birth cohort who will go on to develop vCJD. Alternatively, if a single positive follicle is indicative of an insufficient amount of PrP^{res} to spread and cause disease, the interpretation is that there is no one in the 9160 samples from the 1961–1985 birth cohort who will go on to develop vCJD. The decision between these two interpretations needs to be considered in the context of the relative sensitivities of the different tests that were used, and also in the context of the pathological significance of a small quantity of PrP^{res} in a tonsil. Although all three methods (EIA, IB, and IHC) are based on the recognition of PrP^{res} by specific anti-PrP antibodies, they are qualitatively and quantitatively different. As just a few stained cells can be seen by IHC, it could be argued that it is the more sensitive technique. Conversely, however, as a greater volume of tissue and therefore a larger number of cells can be tested by EIA and IB, it can be argued that they are the more sensitive methods [15]. However, the distribution of PrP^{res} in the tissue is likely to be an important factor in assessing the comparative sensitivities of different tests: when there is a very focal deposition of PrP^{res}, IHC may be assumed to have the advantage.

Therefore, while we cannot say whether the patient from whom this tissue came will go on to develop vCJD, we can be reasonably certain, however, that the patient has not yet developed disease as the codon 129 *PRNP* genotype is MV, and all probable and definite vCJD cases to date have been MM at this loci. There have been four 'possible' cases of clinical vCJD, one of which was MV, but this was not biochemically confirmed and it was in a different birth cohort from the person from whom the tonsil in our study came [30]. Also, the two IHC positives (out of three) from the previous study [26] for which a codon 129 genotype could be determined were *PRNP* codon 129VV [31] and no vCJD cases of this genotype have been reported.

The prevalence in the British population of underlying disease-related prion protein calculated from these findings is, if specimen 38 660 came from a vCJD-positive person, 109 per million for the 1961–1985 birth cohort, with a 95% confidence interval (CI) of 3–608 per million (Table 2), which is not different (exact $p = 0.63$) to the finding of three positives from 10 278 samples for the appendix survey [26]. If tonsil 38 660 did not come from a vCJD-positive person, then the prevalence is 0 per million with an upper 95% CI of 403 for the 1961–1985 cohort and 0 per million for the 1961–1995 cohort with an upper 95% CI of 394 (Table 2), which is not different (exact $p = 0.25$) from the previous study.

It is possible that infection arising from exposure to BSE could cause more than one type of prion disease [32–34]. Strains other than that resulting in vCJD, if they exist, may have markedly different pathogenesis, tissue distributions, and structural forms of PrP^{res}. In addition, it is possible that genetic variability in the population may alter the pathogenesis of vCJD, in that the timing and rate of PrP^{res} in appendix and tonsil tissues may differ between individuals. Indeed, genetic differences may even determine the extent of lymphoreticular pathogenesis [31].

Given that the collection of tonsils in our study has occurred later than the collection of appendix samples in the earlier appendix survey, it is conceivable that tonsils have been collected from infected individuals *further into the incubation period than is the case for* those individuals whose appendices were tested in the earlier survey [26]. Moreover, should the incubation period for prion disease be considerably longer in people with different genotypes, uncertainty about the timing of the appearance of detectable PrP^{res} in these will increase, with concomitant implications for the interpretation of results of PrP^{res} prevalence surveys [6].

Animal experiments have shown that high infectivity, and even disease, can be present in the absence of detectable PrP^{res} [35]. However, this observation cannot be generalized, as PrP^{res} has always been detectable in the lymphoid tissues that have been tested from

vCJD patients [6,25,28]. Data from animal experiments also show 'clearance' of PrP^{res} after inoculation [35,36]. Therefore, the PrP^{res} found in the earlier survey of appendix tissue [26] may conceivably have been transient and eventually cleared without resulting in clinical disease, and therefore the result of the appendix survey result may not be replicable by the current tonsil survey [6].

Although, statistically, the vCJD prevalence estimates in this work do not differ significantly from those obtained by calculating from the previous Hilton study [26], qualitatively they suggest that prevalence estimates may be cautiously lowered. However, in an attempt to provide statistically significant evidence to demonstrate this, a large-scale IHC survey of recently collected appendix tissue specimens for the presence of PrP^{res} is underway.

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Abbreviations

BSE	bovine spongiform encephalopathy
CI	statistical confidence interval
DAB	diaminobenzidine
EIA	enzyme immunoassay
FDC	follicular dendritic cell
H&E	haematoxylin and eosin
HPA	Health Protection Agency
IB	immunoblotting (western blotting)
IHC	immunohistochemistry
PBS	phosphate buffered saline
PMCA	protein misfolding cyclic amplification
PRNP	gene encoding the prion protein
PrP^{res}	disease-related prion protein, specifically the proteinase-K resistant core (it is also referred to in the literature as PrP^{Sc} and PrP^{CJD})
vCJD	variant Creutzfeldt–Jakob disease

Author contribution statement

MM, JL, and SB performed the IHC. JPC was responsible for the tonsil archive. ONG originally initiated the project. MM, SB, and JPC wrote the manuscript.

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研究報告の概要

英国の HPA（Health Protection Agency：健康保護局）が 2009 年の 2 月に公表した報告では、70 歳超の男性血友病患者で臨床症状の出現前の変異型クロイツフェルト-ヤコブ病(vCJD)がほぼ確実な症例について報告されており、また遺伝的にみて vCJD に感染しやすいと考えられるヒトの集団がこれまでに考えられていた範囲よりも広いとの情報から、米国食品医薬品庁(FDA)は米国血漿由来第 VIII 因子(pdFVIII)のレシピエントに vCJD 罹患の危険性があるか再検討することとした。上述の男性血友病患者は死亡時に 70 歳代で、11 年前に英国血漿由来第 VIII 因子製剤での治療を受けており、その製剤は「vCJD に関連している」とされたロットのもの、すなわちドネーション後に確実またはほぼ確実とされた vCJD によって死亡したドナーからの少なくとも 1 回のドネーションを含んだプール血漿から製造された pdFVIII の 1 ロットであった。

FDA は TSEAC の 2009 年 6 月 12 日の会合で、FDA が行った 2006 年 10 月 15 日付のリスク評価「米国内採取血漿から米国承認のもとに製造されたヒト血漿由来第 VIII 因子製剤の使用に伴う vCJD 罹患のリスクの定量的評価　ドラフト」の最新化を提示した。FDA は新たに集積された科学的情報に基づいてこのリスク評価の最新化作業を 2009 年に開始した。この文書は、潜在的 vCJD 罹患リスクの FDA リスク評価文書および評価モデル、ならびに米国内採取血漿から米国承認のもとで製造された血漿由来第 VIII 因子製剤の使用についての 2010 年最新化文書の完全版である。

この文書「米国内採取血漿から米国承認のもとに製造されたヒト血漿由来第 VIII 因子製剤の使用に伴う vCJD 罹患リスクの定量的評価　ドラフト　2010 年最新化版」は、米国血漿から製造されたヒト血漿由来第 VIII 因子(pdFVIII)製剤が用いられた重症の血友病 A(HA)患者および重症のフォン・ヴィレブランド病(vWD)患者における vCJD 原因物質に対する暴露の可能性(確率)とそのレベル、ならびに vCJD に感染するリスクを定量的に推定するものである。

FDA の pdFVIII リスク評価モデルで得られた結果は、米国で製造された pdFVIII からの vCJD 感染のリスクは非常に低いと考えられるが、ゼロではない可能性を示唆している。米国の血漿ドナーについて vCJD 感染リスクの主なソースは、1980 年以降に英国、フランス、またはその他のヨーロッパ諸国に旅行および/または居住していた間の食物を介する暴露である。ドナー排除基準が 1999 年から実施されて vCJD に暴露された人によってドネーションが行われるリスクは低下したが、排除されなかった人もおり、潜在的には vCJD 原因物質を含んでいる血漿がドネーションされる可能性がある。しかし、本評価モデルでは、vCJD に汚染された血漿プールができる可能性は低いことが示唆されている。

ヒト pdFVIII 製剤の製造工程は vCJD 原因物質が存在していたとしても、それを低減させるものと思われるが、製造ステップを経由してどの程度低減されるかは正確には分かっていない。製造における TSE 原因物質のクリアランスは製剤によって異なるものと考えられるが、これまでのところ標準化された研究で測定されたことはなく、それが行われていればより意味のある直接比較ができていたであろう。現在得られている実験研究の結果に基づけば、pdFVIII 製剤では製造工程での vCJD 原因物質の低減が 4 log10(すなわち 10,000 分の 1)となるものと推定されている。製造工程での低減を 4～6 log10 と仮定すると、本評価モデルでは、pdFVIII 製剤を用いて重症血友病 A の治療を受けた患者の 1 年あたりの潜在的リスクは、vCJD の発生率として高い推定値を用い、製剤使用量が多い場合には 15,000 人年に 1 回

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。

2. 重要な基本的注意

(1)略

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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。

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	の感染リスク、vCJD の発生率としてより低い推定値を用い、製剤の使用量が少ない場合には 9,400,000 人年に 1 回のリスクと予測される。現在までに行われたクリアランス研究に用いられた方法、それらの結果自体、および情報のギャップは様々であったので、現時点である特定の製品が他の製品よりもより安全もしくは安全でないと断言することは不可能である。 本評価モデルでの結果は vCJD 原因物質への暴露の可能性があること、また非常に低いながら潜在的感染のリスクがあることを示唆してはいるものの、本評価モデルでは一般性のある vCJD リスクの正確な推定、または個々の患者への実際上のリスクを正確に提示することは不可能である。実際上のリスクは非常に不確実ではあるが、本リスク評価モデルは、感染リスクに影響を及ぼす最も重要な因子が、製造ステップでの vCJD 原因物質のクリアランス、個々の患者がどの程度の量の製剤を用いるか、および英国のドナー集団における vCJD の発生率であることを示している。 我々の評価モデルでの結果は、pdFVIII 製剤による vCJD 感染の実際上のリスクが非常に小さいことを示唆している。米国で vCJD 症例は出ていないが、そのことが将来の何らかの時点で、いくらかのレシピエントで vCJD への暴露が起こり vCJD がもたらされる可能性を排除するものではない。	

報告企業の意見	今後の対応
血漿分画製剤は理論的なvCJD伝播リスクを完全には排除できないため、投与の際には患者への説明が必要である旨を2003年5月から添付文書に記載している。2009年2月17日、英国健康保護庁(HPA)はvCJDに感染した供血者の血漿が含まれる原料から製造された第Ⅷ因子製剤の投与経験のある血友病患者一名から、vCJD異常プリオン蛋白が検出されたと発表したが、弊社の原料血漿採取国である日本及び米国では、欧州滞在歴のある献（供）血希望者を一定の基準で除外し、また国内でのBSEの発生数も少数であるため、原料血漿中に異常型プリオン蛋白が混入するリスクは1999年以前の英国に比べて極めて低いと考える。また、製造工程においてプリオンが低減される可能性を検討するための実験を継続して進めているところである。	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。

DRAFT

A 2010 Update of the 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 6, 2010

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EXECUTIVE SUMMARY

A February 2009 report from the Health Protection Agency of the United Kingdom (UK) of a probable case of pre-clinical variant Creutzfeldt-Jakob Disease (vCJD) infection in a man over 70 years of age with hemophilia and recent information on broader genomic susceptibility to vCJD of human population prompted the U.S. Food and Drug Administration (FDA) to re-examine the potential vCJD risk for recipients of US-sourced pdFVIII. The man, who was in his 70s at death, had been treated 11 years earlier with UK-sourced plasma-derived Factor VIII (pdFVIII) from a "vCJD-implicated" lot, i.e., a lot of pdFVIII manufactured from pooled plasma containing at least one donation from a person who later died of confirmed or probable vCJD.

FDA presented an update of its risk assessment "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) at the June 12, 2009 Meeting of TSEAC. The FDA began updating the risk assessment in 2009 based on new accumulating scientific information. This document is the completed 2010 update of the FDA risk assessment documents and model of potential vCJD risks and the use of human plasma-derived factor VIII manufactured under United States (US) license from plasma collected in the US.

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 June 2010, 221 cases have been reported worldwide, with 174 cases in the U.K. Since December 2003, there have also been four 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 four red blood cell recipients who probably became infected with the vCJD agent after transfusion, three developed vCJD and died from the disease. The third died of an unrelated illness. 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 "A 2010 Update of the 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 from US plasma. 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 severe 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 4.5 vCJD cases per million in the UK.

Running the model with this vCJD case prevalence estimate (~4.5 per million) produces an estimate suggesting that, on average, there was a 0.03% 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 5th and 95th 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 2.3% 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 Case Prevalence estimate (~4.5 in 1 million) indicated that, on average, there is a 0.03% (5^{th} - 95^{th} perc: 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 given the range of predicted answers 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, 0.03% 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 2.3% (5^{th} - 95^{th} perc: 0 % - 8.2 %) 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 and Prevention (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 Factor (LRF)	
				Model Output for LOWER vCJD Case Prevalence of ~4.5 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 [b] (5th - 95th perc)**	**Mean potential vCJD risk per person per year [a] [b] (5th - 95th perc)**	**Mean potential vCJD risk per person per year [a] [b] (5th - 95th perc)**
Prophylaxis	No Inhibitor	**578**	**157,949 IU#[d]** (21,000 , 382,000)	1 in 4.0 million (0-0)[c]	1 in 63,000 (0- 1 in 13,000)
	With Inhibitor – No Immune Tolerance	**63**	**190,523 IU#[d]** (27,000, 448,000)	1 in 3.4 million (0-0)[c]	1 in 53,000 (0- 1 in 11,000)
	With Inhibitor – With Immune Tolerance	**62**	**558,700 IU#[d]** (33,000, 1,593,000)	1 in 1.1 million (0-0)[c]	1 in 18,000 (0- 1 in 3,700)
Episodic	No Inhibitor	**946**	**85,270 IU#[d]** (46,000, 245,000)	1 in 7.1 million (0-0)[c]	1 in 115,000 (0- 1 in 24,000)
	With Inhibitor	**151**	**160,458 IU#[d]** (5,000, 489,000)	1 in 4.0 million (0-0)[c]	1 in 61,000 (0- 1 in 13,000)

Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 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 95% 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 95% 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 2,600 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 41 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 Factor (LRF)	
			Model Output for LOWER vCJD Case Prevalence of ~4.5 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**	**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 2,600 years (0-0)[d]**	**1 in 41 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] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 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 95% 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 95% 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 and Prevention (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 vCJD Case Prevalence estimate of ~4.5 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 23,000 years. At the HIGHER vCJD Infection Prevalence estimate, the average potential vCJD infection risk for this population is 1 infection in 360 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.

				4 -6 Log_{10} Reduction Factor (LRF)	
				Model Output for LOWER vCJD Case Prevalence of ~4.5 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 (5th - 95th perc)[c]**	**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] (9900, 454300)[#]**	**1 in 3.8 million (0-0)[e]**	**1 in 59,000 (0 - 1 in 12,000)**
	Episodic	**60**	**11,045 IU[d] (1020, 34350)[#]**	**1 in 56 million (0-0)[e]**	**1 in 830,000 (0 - 1 in 210,000)**
ADULT vWD (> 15 yrs of age)	***Prophylaxis***	**73**	**186,880 IU[d] (17000, 540000)[#]**	**1 in 3.4 million (0-0)[e]**	**1 in 53,000 (0 - 1 in 11,000)**
	Episodic	**78**	**86,923 IU[d] (2200, 240000)[#]**	**1 in 7.1 million (0-0)[e]**	**1 in 110,000 (0 - 1 in 23,000)**

[#] Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[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] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 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 95% 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 95% 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 Factor (LRF)	
			Model Output for LOWER vCJD Case Prevalence of ~4.5 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	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	27.5 million IU[d #]	1 in 23,000 years (0 - 0)[e]	1 in 360 years (0 - 1 in 74)

[a] Mean Total quantity FVIII used by all patients was incorrectly reported as 29.9 million IU in the FDA 2006 risk assessment, the value of 27.5 million IU is the correct value

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] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 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 95% 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 95% 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.

Results from our model suggest that the actual risk of vCJD infection from pdFVIII is likely to be very small. The absence of cases in the US 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

In February 2009 the Health Protection Agency of the United Kingdom (UK) reported a probable case of pre-clinical variant Creutzfeldt-Jakob Disease (vCJD) infection in a man over 70 years of age with hemophilia (http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681). Post-mortem examination of the brain found no neuropathological changes suggestive of vCJD, however, examination of the spleen revealed abnormal accumulation of prion protein (PrP^{res}) typical of vCJD. The man, who was in his 70s at death, had been treated 11 years earlier with UK-sourced plasma-derived Factor VIII (pdFVIII) from a "vCJD-implicated" lot, i.e., a lot of pdFVIII manufactured from pooled plasma containing at least one donation from a person who later died of confirmed or probable vCJD.

The recent vCJD infection case of the hemophilia patient and newly emerged information on broader genomic susceptibility to vCJD of human population prompted the U.S. Food and Drug Administration (FDA) to re-examine the potential vCJD risk for recipients of US-sourced pdFVIII. FDA presented a previous version of a risk assessment model at the December 15, 2006 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for vCJD risk associated with patients with a severe form of hemophilia A (HA) or von Willebrand disease (type-3 vWD) who have used pdFVIII product manufactured in US-licensed facilities. The document of the risk assessment "*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*" was posted on the FDA website. The FDA began updating the risk assessment in 2009 based on newly accumulated scientific information. The updates of the risk assessment were presented at the June 12, 2009 meeting of TSEAC. This document provides a 2010 update of the FDA risk assessment of potential vCJD risks and 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 and this aspect was not incorporated into the 2010 update. 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 other 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 collected in the US. Risk for these products was estimated for the baseline year of 2002, when FDA current guidance for donor deferral for vCJD risk was published, 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

Variant Creutzfeldt-Jakob Disease and potential risk associated with human plasma-derived product

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 June 2010, 221 cases have been reported worldwide, with 174 cases in the UK. Since December 2003, there have also been four 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 four red blood cell recipients who probably became infected with the vCJD agent after

transfusion, three developed vCJD and died from the disease, one 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. In 2004, UK authorities 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 June, 2010, reported 174 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 July 2010 France has reported 25 cases of confirmed or probable vCJD (www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html). 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.

In 1999, prior to the identification of transfusion-transmitted vCJD, FDA recognized a potential though unknown risk of transmitting vCJD by contaminated blood products. Therefore, consistent with advice from TSEAC, FDA recommended precautionary deferrals of blood and plasma donors who had traveled or lived for six 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. FDA added certain other measures to reduce potential risk, such as deferring any donor with a history of blood transfusion in the UK after 1979 (http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095138.htm; http://www.fda.gov/BiologicsBloodVaccines/SafetyAvailability/BloodSafety/ucm095143.htm). Taken together, these steps were estimated to have excluded donors representing slightly more than 90% of the potential 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 since 1980. FDA has recently issued revised guidance containing such recommendations (FDA 2010).

Because BSE has been detected in so few US cattle (only three reported cases: two in US-born cattle and one in a cow imported from Canada [http://www.ars.usda.gov/research/publications/publications.htm?SEQ_NO_115=197033]), and because none of the three cases of vCJD recognized in the US appears likely to have resulted from exposure here (two cases in long-time UK residents and a third in a recent immigrant from Saudi Arabia), the risk that US plasma donors might have acquired vCJD infection from US beef is thought to be extremely low. (Because the likelihood of exposure of US donors to the BSE agent in US beef products was judged to be so much lower than likelihood of exposure in UK, its estimated contribution to overall risk seems negligible and—while not ignored in developing FDA Risk Assessments—was not included in the model summarized here.) However, it is possible that a few US donors might have been exposed to the BSE agent during travel or residence in the UK, France, or certain other countries of Europe; such donors are at an uncertain but increased risk for vCJD. A subset of such vCJD-infected donors might have contributed to plasma pools used to manufacture pdFVIII in the US. The FDA-recommended donor deferral policy probably eliminates most of the risk associated with vCJD-infected individuals; however, there could be residual risk from eligible donors who were nonetheless infected during brief stays in foreign countries (Yamada 2006) or from donors who should have been deferred by the screening process, but, for an unknown reason, were not.

Hemophilia A, von Willebrand disease 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 less than one percent (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.
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 and vWD 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 for HA 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 baseline 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. The probable transmission of vCJD to four recipients of red blood cell products donated by donors later diagnosed with vCJD in the UK had raised concern that vCJD might be transmitted via plasma-derived products. The most recent reported vCJD infection of a hemophilia A patient made the theoretic risk a more probable risk.

Human vCJD was first reported in the United Kingdom in 1996 (Will *et al* 1996). As of June 2010 over 221 cases, 174 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, with bovine spongiform encephalopathy (BSE) agent present in 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 15 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.

Transmission of vCJD in the United Kingdom via blood, blood products and plasma-derived products

Secondary transmission of vCJD has likely occurred on several occasions for transfusion of blood and blood products; and plasma-derived products. As previously mentioned, the UK Health Protection Agency (2009) reported a probable case of pre-clinical vCJD infection in a man over 70 years of age with hemophilia in February 2009. Post-mortem examination of the brain found no neuropathological changes suggestive of vCJD, however, examination of the spleen revealed abnormal accumulation of prion protein (PrP^{TSE}) typical of vCJD. This was the first report of discovery of abnormal vCJD prion protein in a patient with hemophilia. To date, no hemophilia or bleeding disorder patients have been diagnosed with or died from clinical vCJD.

As of June 2010 four cases of transfusion-transmitted vCJD have been identified in the UK. The first case was announced 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. A second case was announced in July 2004 and occurred in a patient who 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. In January 2007, the fourth probable transfusion-transmitted vCJD case had been reported; the patient was diagnosed about nine years after receiving a blood transfusion from the same blood donor who was also associated with one of the previously identified cases. 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 cases that were reported after red blood cell or plasma-derived product transfusions; however, given the circumstances, the probabilities that either a single, or, particularly, five such events are not associated with transfusion are small. 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. As Llewelyn *et al* (2004) pointed out in their publication discussing the first presumed blood cell transfusion-transmission 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."

Potential vCJD risk for travelers with a 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 of specified risk materials, and others have been instituted 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 older than 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.

US travelers to and residents of the UK, France and other countries in Europe during the period of BSE pandemic may have been exposed to the BSE agent through dietary sources and 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 trade and export of cattle products from one country that are 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, the 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 focuses 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 focuses on the time (cumulatively 5 years or more) that a person lived in these countries from 1980 through the present. This deferral policy likely reduces the risk of vCJD transmission via blood and plasma donations from potential infected donors.

Three cases of vCJD in US residents who were likely infected outside the US

In 2002, the first case of vCJD was reported in the United States in a 22-year-old woman who was living in Florida and is believed to have become infected with vCJD during extended 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.

The third patient 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. The patient's onset of symptoms occurred in Spring 2006. The 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, this case likely attributed to consumption of BSE-contaminated cattle products in Saudi Arabia.

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 and Prevention (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 transmission of vCJD via blood transfusion or plasma-derived products is no longer theoretical but a real possibility. Transmission of vCJD via transfusion of red blood cell products (Llewelyn *et al* 2004) and a plasma-derived product (http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733818681) have likely occurred.

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 frequently 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 has been observed 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 FDA Transmissible Spongiform Encephalopathies Advisory Committee (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.

The FDA assumes persons with *PRNP*-MV and VV genotypes are all equally susceptible to vCJD infection as MM genotype and that they might also progress to develop clinically symtomatic vCJD. The MM, MV and VV genotypes are thought to comprise approximately 40%, 50% and 10% of the population, respectively. The FDA updated the risk assessment for potential vCJD infection for recipients of US pdFVIII and presented the assessment at the June 2009 Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) meeting and incorporated the assumption of susceptibility of all genotypes into this 2010 risk assessment model.

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 contributing considerable uncertainty to estimating vCJD risks.

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.

Potential vCJD risk for use of US pdFVIII products may be expected to vary, to some degree, from year to year since 1980. In this risk assessment, the potential vCJD risk associated with pdFVIII products was estimated for the baseline year of 2002, but the results and conclusions are likely to reflect the current risk.

This section of the document provides a general description of modeling approaches, rationale, input data, assumptions and results of the model. Additional technical details on the model and calculation are provided in Appendix A in sections under A-IV. The section titles and numbers used in this document are consistent with those used in Appendix and model spreadsheets.

Overview of Model

Module 1 – Estimation of the Prevalence of vCJD in the UK. This module estimated the vCJD prevalence in the UK 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 estimate for UK vCJD case prevalence: generated based on epidemiological modeling of clinical cases (Clarke and Ghani 2005), and adjusted to include the MV and VV genotypes as subpopulations that are equally susceptible to vCJD infection as MM genotype and that might also progress

to develop clinically overt vCJD. The mean estimate is approximately ~4.5 cases per million persons.

- A tissue surveillance-based estimate for UK vCJD infection prevalence: generated using data from a tissue surveillance study (Hilton *et al* 2004). The mean estimate is 1 case per 4,225. Most of tissue samples examined were from patients at the age group of 20-29 years old.

Prevalence of asymptomatic vCJD in UK in 2002 was estimated using the above data and adjusted for age and susceptibility of population groups.

Module 2 –vCJD Prevalence in US Plasma Donors and Plasma Pools. This module estimates the number of US plasma donors that may potentially be infected with vCJD, the percentage or number of plasma pools containing vCJD agent, and the amount of vCJD infectivity in individual pools. The model assumed that the major source of vCJD in the US would likely be associated with plasma donors who have a history of travel and residency in the UK, France or other countries in Europe since 1980 who may have had dietary exposure to the BSE agent during their stay. This module uses blood donor 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), or during Military service when posted on or residing near military facilities in Europe; and
- Transfusion with blood collected in Europe, or Euroblood.

vCJD prevalence in US plasma donors was estimated based on vCJD prevalence in the UK, number of US plasma donors who have history of travel or residence in BSE countries and relative exposure risk of those at-risk US plasma donors compared to UK residents. US plasma donors who were potentially at risk for vCJD were characterized by:

- Age
- *PRNP* genotypes
- Country, year and duration of travel or residence

Other factors were also included in the calculation of vCJD prevalence of US plasma donors and plasma pools:

- Effectiveness of donor deferral policies
- The time period during the course of disease when blood of an infected person contain vCJD infectious agent
- Type of plasma pool (source or recovered), number of donors per plasma pool, and
- Age specific rate and frequency of plasma donation,

The amount of infectivity in an infected plasma pool was calculated based on the estimated amount of infectivity in a unit of plasma donated by an infected person, the size of plasma pool, and the likely number of infected donations per plasma pool.

Module 3 - pdFVIII Manufacturing and Processing. This portion of the model calculated the quantity of vCJD agent in pdFVIII products made from an infected plasma pool based on:

- Initial quantity of infectivity (i.v. ID_{50}) present in the infected plasma pool
- Reduction in the quantity of potential vCJD agent during manufacture, and
- Total yield and number of international units (IU) of pdFVIII produced from plasma pool.

Considering the uncertainty in the degree of infectivity clearance that can be achieved during various pdFVIII manufacturing processes, this risk assessment models two levels of potential clearance in infectivity, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

Module 4 - Utilization of pdFVIII by Hemophilia A (HA) and von Willebrand Disease (vWD) Patients. The potential exposure of an individual HA patient or vWD 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 HA patient is dependent on the severity of the disease and the treatment regimen and was estimated using data from a Centers for Disease Control and Prevention and Prevention (CDC) sponsored study in 6 states 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:

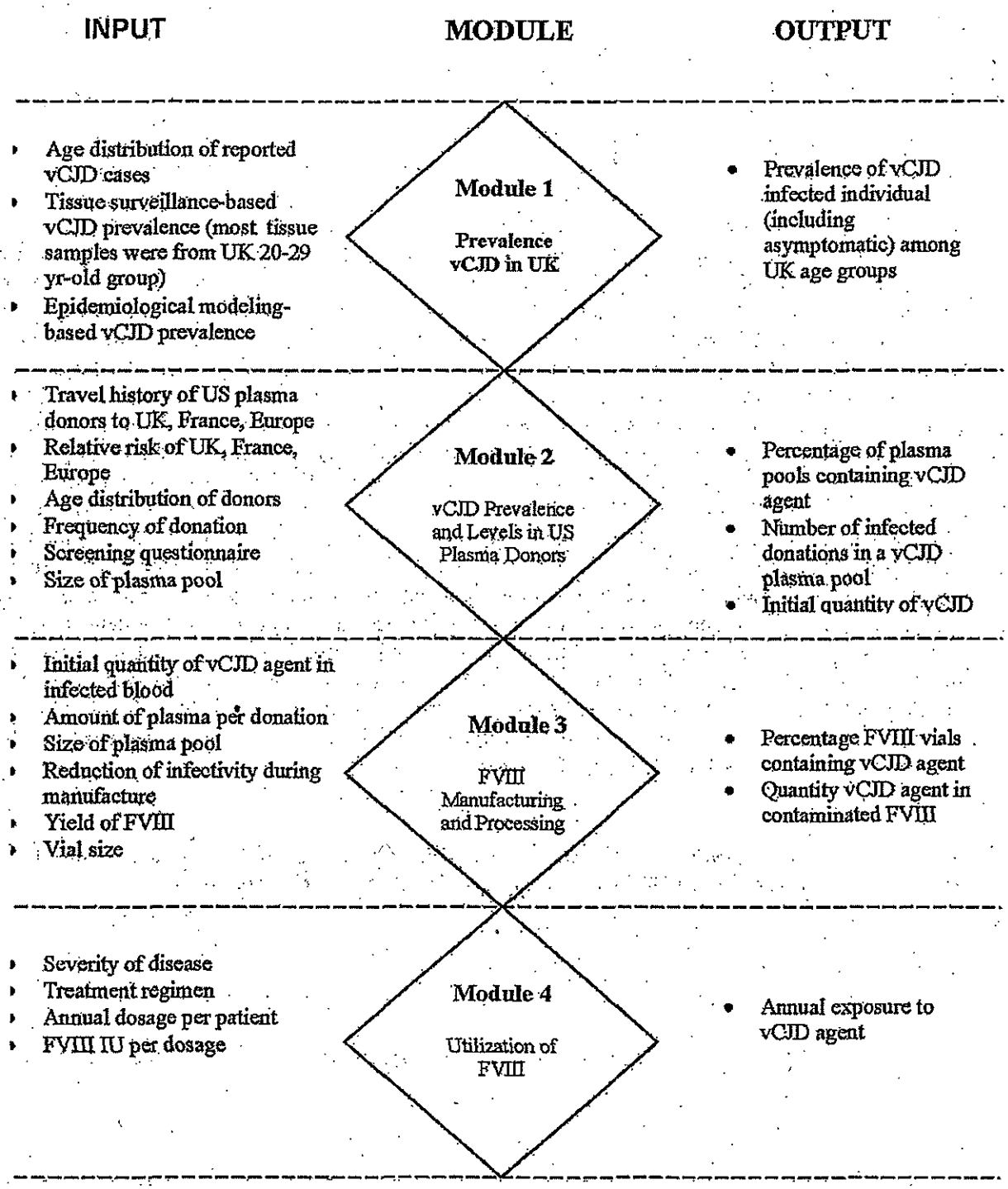
- Prophylaxis
- Prophylaxis plus inhibitor
- Prophylaxis plus inhibitor and immune tolerance
- Episodic
- Episodic plus inhibitor

vWD for adult (≥15 yrs of age) and young (≤15 yrs of age) persons, including those in either clinical treatment group:

- Prophylaxis
- Episodic.

Most of the model inputs are statistical distributions representing the variability and uncertainty associated with the input variables. In general, we used a point estimate if no information was available that could be used to quantify the variability and uncertainty; a uniform distribution, consisting of a minimum and maximum value, when there was only enough information to define a range; a triangular distribution, consisting of a minimum, most likely, and maximum value, when there was enough information to define a range and a most likely value. We used other more sophisticated parametric distributions when there was enough data with which we could fit a statistical distribution. In other cases, we used point estimate for sets of correlated input variables such as donation rates by individual age group and percentage travel by destination. Applying statistical distributions to these variables would greatly complicate the model and likely require several extra days to compute the results and we believe point estimates give a reasonable representation of the input variables. However, we acknowledge that using point estimates may underestimate the uncertainty associated with the input variables.

Figure 1. Model of Exposure Assessment



IV. A. Estimation of vCJD Prevalence in the United Kingdom (Module 1)

This module estimates the vCJD prevalence in the UK population by age and genotype. The UK vCJD prevalence was used as the basis for calculation of vCJD prevalence of US plasma donors in the following module of the model.

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. The number of cases per year in the UK reached a maximum of 28 in the year 2000, and since then has been continuously declining with 3 confirmed or probable cases identified in year 2009. This section of the risk assessment estimated the vCJD prevalence in the UK in 2002, but again, assumes the estimated risk would be valid for current day in the year 2010 .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 the prevalence among US donors the UK vCJD prevalence was adjusted based on the time spent in the UK, the year of travel and age of the donor. The number of asymptomatic vCJD cases 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 them. The prevalence of asymptomatic vCJD infections in the UK in 2002 was estimated in the FDA model using two different approaches based on two different data sources. 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. A check panel is set up on the worksheet, "Model Control", which allows us to switch UK vCJD prevalence estimates from one to another. The discrepancy between the two estimates reflects the limitation on the current knowledge of the disease.

IV. A. 1. UK Asymptomatic vCJD Infections Estimated using Epidemiological Modeling Results (Clarke and Ghani 2005) and Adjusted for All Three Genotypes

IV.A.1. a. Estimation of the Number of Asymptomatic vCJD Infections in the UK in 2002

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 a mean 70 future vCJD cases (90% CI: 10-190 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 current vCJD cases and potentially incubating vCJD infections in the year 2002.

In the 2006 version of the model we added 32 total cases diagnosed in years 2002-2003 and the estimated 70 vCJD cases for years 2004-2008 (Clarke and Ghani 2005) to estimate the number of cases in the UK for the years 2002 – 2080. We assumed that all clinical cases predicted to occur after 2002 were incubating in 2002, thus, representing the number of vCJD infections among the total UK population in 2002. This prediction did not account for potential asymptomatic vCJD infections from PRNP-129 MV and VV genotypes, because all reported clinical vCJD cases had been in persons with the PRNP-129 MM genotype.

As mentioned earlier, recent findings suggest that it is now more reasonable to assume that the entire general UK population is at risk for vCJD infection, and this assumption has been incorporated throughout the FDA 2009 updated draft risk assessment presented at the June 2009 TSEAC meeting. Our 2010 risk assessment also assumes all genotypes to be equally susceptible to vCJD infection, and vCJD infections among PRNP-129 MV and VV genotypes might eventually progress to develop clinically overt vCJD. Therefore, predicted vCJD clinical cases for the whole population of three genotypes from 2002 to 2080 was derived by multiplying predicted number of cases for MM genotype with a factor of 2.5 (times that size of total population compared to the size of MM sub-population). Therefore, the FDA model estimated an average of 255 cases ((32+70) x 2.5=255) of asymptomatic vCJD infections for the year 2002 wth a 5th percentile of 105 cases ((32+10) x 2.5=105) and 95th percentile of 555 cases ((32+190) x 2.5=555). 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 4.5 vCJD infections per million population (255 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-MM genotype (Health Protection Agency, 2006)				**Estimation of future UK vCJD cases-MM genotype** (Clark and Ghani 2005)		**FDA model: Estimation of UK vCJD cases for years 2002 - 2080-All three genotypes**
Year(s)	**2002**	**2003**	Total		**2004 - 2080**		**2002 - 2080**
Number of vCJD cases	16	16	32		70 (10 – 190)		255 (105 – 555) Mean =(32+70)x2.5=255 5th=(32+10)x2.5=105 95th=(32+190)x2.5=555

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. However, because no cases of clinical vCJD have been identified in individuals with non-MM genotypes, it is still 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.

Assumptions used in the model:

- All genotypes are equally susceptible to vCJD infection, and vCJD infections among PRNP-129 MV and VV genotypes might eventually progress to develop clinically overt vCJD.
- All vCJD cases that occur after 2002 are incubating in year 2002

IV.A.1. b. Age Distribution of Asymptomatic vCJD for All Three Genotypes

The number of asymptomatic vCJD cases in the UK derived from epidemiological modeling results above is the average number of cases for entire population. In order to extrapolate the prevalence to other age groups, this section of the model calculated the age distribution of UK subpopulation who are infected with vCJD and asymptomatic. The age distribution of individuals who are infected with vCJD and asymptomatic was derived from the data on the number of persons diagnosed with vCJD from different age groups, and the assumptions on incubation period of the disease. The distribution of age at time of initial infection is calculated by left shifting the distribution of age at diagnosis by 15 years, which is the estimated average incubation period for the MM genotype. The model assumed that this distribution of age at time of initial infection is applied to all three genotypes. The distribution of age at time of diagnosis for *PRNP*-129 MV and VV genotypes was generated by right shifting the distribution of age at time of diagnosis for MM by an extra incubation period needed for MV and VV compared to MM genotype. The model used a gamma distribution with mean of 15 years, 5th and 95th percentile of 5 and 35 years to represent the incubation period for persons with the MM genotype. The estimation of incubation periods for people with MV and VV genotypes remains complicated and more uncertain than for persons with MM genotype, because so for there has been no clinical cases or deaths from vCJD reported from

non-MM genotypes. So it is not possible to precisely estimate the duration of the incubation period of vCJD in non-MM persons. Given this considerable uncertainty, we made simplifying assumptions that the incubation period for non-MM is 20-year longer than MM represented by a Gamma distribution with mean of 35 years, 5th percentile of 25 years, and a 95th percentile of 55 years. The high value of 55 years (95th percentile) was estimated based on the maximum incubation period for kuru (Collinge 2006). The values of incubation period for MV and VV genotypes are randomly drawn from this distribution.

For any given age category, the probability that an individual is infected and asymptomatic can be calculated by multiplying the cumulative probability that they are infected (from the distribution of age at time of initial infection) by the cumulative probability that they have not been diagnosed (from the distribution of age at time of diagnosis). These probabilities are then normalized to sum to one to the age distribution of individuals who are infected and asymptomatic.

Assumptions used in the model:

- The distribution of age at initial infection is the same for all genotypes
- Genotypes, MM, MV and VV represent 40%, 50% and 10%, respectively, of the total donor population

IV.A.1.c. Prevalence of Asymptomatic vCJD in the UK by Age and Genotype

Combing the data derived from the previous two sections, this section of the model estimated the prevalence of asymptomatic vCJD in each UK age subpopulations with specific genotype.

IV.A.2. UK vCJD Prevalence derived from a Tissue Surveillance study (Hilton *et al* 2004)

IV.A.2.a. UK Asymptomatic vCJD Prevalence of 20-30 years Age Group

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. However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be considered. 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 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. The 3 positive samples were also from this age group. After adjustment correcting for sampling bias we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million for the 20-29 year old age group (see appendix for detailed calculations).

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, the prevalence of infection might have been underestimated 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. Second, the study design did not permit an estimate of specificity of the method or an independent confirmation of results because it did not examine a large number of similarly obtained appendices from a non-BSE-epidemic country. Therefore, 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."

Module 2 IV.B-IV.D. Potential vCJD Risk for US Plasma Donors and Plasma Pools

This module estimates the annual numbers of US plasma donors who were at vCJD risk, plasma donors who might be infected and contain vCJD infectious agents at the time of donation, and plasma pools used to manufacture pdFVIII that might potentially contain a donation from an

infected plasma donor. This module also estimates the potential quantity of vCJD agent that might be present in a positive.

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.

IV. B. Estimation of vCJD Prevalence in US Plasma Donors and Plasma Pools

IV.B.1. Annual Number of Plasma Donors

This section of the model calculates the annual number of donors who might have contributed plasma used to manufacture plasma-derived FVIII. The calculation was based on the market data for FVIII products, estimated yield of FVIII per unit plasma, donation rate and amount of plasma per donation. The donors were grouped by age and type of donation (source or recovered plasma).

Age is an important factor in estimating potential vCJD risk for US plasma donors. In the US the majority of donors are less than 40 years of age, and since vCJD primarily affects younger persons (median age of 28 yrs for clinical vCJD) The donor population is at particular risk for vCJD. Infected donors, who are asymptomatic at the time of donation, may unknowingly transmit the the infection to recipients (Table 4.3). The FDA model is organized by age groups of 18 and 19 yr olds, 10-14, 15-29, etc. (by five yr age groups to age 69) as "bins". In each of these bins, donors will be further categorized by type of donation (source or recovered), country of travel, duration and year of travel for specific vCJD prevalence (or relative risk).

Two different types of plasma are used in the 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 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). Larger pool size increases the chance that a plasma pool may contain a donation from an infected donor. However, blood deferral policies instituted beginning in 1999 are believed to have reduced the risk of vCJD donations by more than 90%. In addition, 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 they might contribute multiple donations to a single plasma pool or donate to multiple pools. Additionally, Source Plasma donors are usually younger than blood (recovered plasma) donors (see percentages of donors for Source

and recovered plasma donors by age group in **Table 4.3**). Because of their younger age demographic, Source Plasma donors are likely to be more susceptible to vCJD infection. 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 donors and plasma pools separately, and considered the factors that may result in different risk for pdFVIII product made from each of the two types of plasma. Besides, Source Plasma donors are thought to travel less, so presumably their vCJD risk may be somewhat lower than that of blood donors. However, because travel data for source plasma donors is not available, FDA risk assessment used travel data of blood (recovered plasma) donors for source plasma donors, which may lead to slight overestimate of the risk for source plasma donors.

Table 4.3. Reported vCJD cases in the UK and Percentage of US Source Plasma and Blood (recovered plasma) Donors by Age Group

Age group	<10	10-14	15-17	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.B.2. Annual Number of Plasma Donors Potentially Infected and Whose Blood May Contain Infectivity

The purpose of this section of the model is to estimate the prevalence of vCJD in US donors and the probability that a plasma pool may contain a donation (donations) from a vCJD infected donor with infectious agent in their blood at the time of donation.

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 numbers of US plasma donors who might have been infected and contain vCJD infectivity in blood are calculated separately in the worksheets, "IV.B.2.a. Travel-UK", "IV.B.2.b. Travel-FR", "IV.B.2.c. Travel-EU", "IV.B.2.d. Military", "IV.B.2.e. Euroblood" for different exposure sources; then summarized and summed to yield an annual total of US plasma donors who might have infected and contain vCJD infectivity in blood in worksheet "Model-IV-Exposure Assessment".

The percentages of blood donors with a history of travel or residency in the UK, France and other European 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 of at-risk donors was calculated by destination (e.g., the UK, France or other European countries) and duration of travel, further adjusted for year of travel. In the 2009 version of FDA's model, the percentage of at-risk donors is further adjusted for different *PRNP*-129 genotypes. Travel data used in this risk assessment may not accurately represent the travel pattern of source plasma donors, who are likely younger and travel less frequently than the blood donors. However, no travel data for source plasma donors is available.

The model estimated the annual number of plasma donors who might have been exposed to vCJD and infected during travel or residence in BSE countries. For US donors with a history of travel to the UK the vCJD prevalence was derived from the vCJD UK prevalence and adjusted for duration of time that donors spent in the UK, and the years of travels. The magnitude of vCJD risk is assumed to be proportional to the accumulated time spent in the UK and correlated with the magnitude of BSE epidemics at the year of travel. Calculation of the potential vCJD risk for donors who traveled to France was estimated relative to the risk for travel to the UK (or the relative risk) based on the amount of beef that France imported from UK, the number of domestically acquired vCJD cases in France, and other factors. The relative risk of vCJD in France was assumed to be 0.05 times that for the UK. 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 by multiplying the UK vCJD prevalence by either 0.05 for vCJD prevalence of France or 0.015 for vCJD prevalence of other European countries. The risk for US plasma donors is further adjusted to account for factors such as the duration of time that donor spent in the BSE countries, and the year of travel.

Then, the model derives an estimate of the number of infected donors who may actually have vCJD infectious agent in blood at the time of donation. The model assumes that infectious vCJD agent most likely present in blood during the later 75% of the incubation period (minimum=50%, maximum=90%) and was represented by a triangular distribution with values of (50%, 75%, 90%). This assumption was based on the results from recent findings from studies in animal models (Brown 2007).

The model further incorporates the risk reduction effect of FDA donor deferral policies, implemented beginning in 1999 and last revised in 2002. Current donor deferral policy defers donors who:

- Traveled to or resided in the United Kingdom from 1980 – 1996 for $\geq$ 3 months
- Traveled to or resided in France - since 1980 for $\geq$ 5 years

• Traveled to or resided in other countries in Europe – since 1980 for ≥ 5 years (does not include source plasma donors)
• Were US Military personnel or their dependents – deployed in UK or other countries in Europe since 1980
• Were Euroblood recipients in US – that received blood collected from donors in Europe

This geographic deferral policy removes donors with a history of extended travel or residence in the UK and other countries in Europe since 1980. It is believed that these policies are likely to reduce the possible vCJD risk from plasma donors by 85- 90% and this range was represented in the model using a uniform distribution (0.85, 0.99). It is impossible to estimate the efficiency of donor deferral directly based on the number of donors who present for donation at a blood center and are deferred on site, because a substantial, unknown number of potential donors likely self-defer and do not present to donate. The efficiency of donor deferral used in this model was extrapolated from the efficiency for other transfusion transmitted diseases, such as HIV, HBV and HCV etc, based on high risk behaviors such as intravenous drug use and others. This extrapolation is thought to reasonably approximate the expected deferral rate, however it may not accurately reflect the efficiency of donor deferral for vCJD risk and represents a source of uncertainty. For example, social stigma and lower social acceptability of high risk sexual behaviors and intravenous drug use, which are associated with higher rates of HIV, HBV and HCV, might discourage some potential donors from responding truthfully to screening questions on the donor questionnaire. Accordingly, the rate of 'true' response to screening questions for potential donors with these behaviors, and thus, efficiency of donor deferal might be lower than for questions concerning travel history that may trigger geographic deferral for vCJD risk.

There is a small chance that infected donors might still donate for two reasons. First, donor screening based on questionnaire is inefficient and subject to bias. Some donors with a history of an extended period of travel or residence in BSE countries may not be indentified by questionnaire screening because of recall failure, recall errors, or other reasons. Other sources of bias such as missing data, misunderstanding or mis-comprehension of questions or false reporting can introduce further inaccuracies in screening. Second, some donors may have been infected while on a short stay in a BSE country that does not meet the guidance deferral criteria, and thus, would not be deferred from donating plasma.

Assumptions used in the model:

- The relative risk for the UK , France and the other European countries is 1, 0.05 and 0.015, respectively
- The risk of vCJD exposure is cumulative, proportional to the duration of stay or time spent in the BSE countries, for instance a person who lived in the UK for one year has one-fifth the risk of a donor who spent five years
- The risk of vCJD exposure is correlated with the magnitude of the BSE epidemic at the time of travel or stay
- The vCJD agent is assumed to appear in the blood of infected persons most likely after 75% of the incubation period of the disease has elapsed, with a range between 50% to 90% elapse of the incubation period.
- Efficiency of donor questionnaire to identify at-risk donors ranges from 85% to 99%.

IV.B. 2.a. US plasma donors with History of Travel to the UK: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

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 174 cases as of June 2010), 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.

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, duration and year of travel, genotype, and estimated the number of donors, probability of an individual being infected, probability of infected individual containing vCJD infectious agent in the blood and potential number of donors infected and whose blood may contain vCJD agent at the time of donation for each group.

IV. B. 2. b. US Plasma Donors with a History of Travel to France: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

Donors who traveled to France are potentially at risk but that risk is likely significantly lower than that for travel to 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 25 cases of vCJD (www.invs.sante.fr/display/?doc=publications/mcj/donnees_mcj.html) supporting the notion that there may be vCJD infection risk for US donors who may have traveled to or resided in France since 1980. 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 fewer domestically-acquired vCJD cases. Therefore, the risk (prevalence) for vCJD for travel or residence in France was assumed to be 0.05 times that for travel to the UK.

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."

IV.B. 2. c. US Plasma donors with history of travel to other Countries in Europe: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

Donors who traveled to other countries in Europe (other than the UK or France) are potentially at risk of vCJD but that risk is likely significantly lower than that for travel to the UK or France. Other countries in Europe 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 risk (prevalence) for vCJD for travel or residence in France was assumed to be 0.015 times that for travel to the UK. 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 was 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.

FDA guidance (2002) indicates that for donors with a history of travel to other countries in Europe (other than the UK and France) "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 other 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 "other 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).

IV. B. 2. d. US plasma Donors Deployed by the Military in the UK or Other Countries in Europe: Annual Number Potentially Infected and Whose Blood may Contain vCJD Agent

For the purposes of this risk assessment we assumed that military personnel or dependents who have been deployed to US military bases in the UK, France and other European countries during the period from 1980 through 1996, might have been exposed to the BSE agent and infected 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.

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)". FDA has recently issued revised guidance containing such recommendations (FDA 2010).

IV. B. 2. 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 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.

IV. B. 2. f. Total Number All Plasma Donors who may Potentially be Infected with vCJD and the vCJD Agent may be Present Through All Sources of Exposure

This portion of the model sums the total number of all potential US donors that may have been infected with vCJD from different exposure 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 Euroblood. Furthermore, the model estimates the number of total US donors potentially infected with vCJD and in whose plasma the vCJD agent may be present.

IV. B. 3. Annual Number of All US Plasma Donors Potentially Infected and Whose Blood may Contain vCJD Agent 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 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 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 who 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 donor vCJD 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 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.

Model Results forModule 2: vCJD Risk of US Plasma Donors

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.

The predicted 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 are shown in Table 4.4 (below). The estimated mean number of US donors who potentially donated plasma containing infectious agent is approximately 0.02 donors per year based on calculations using a vCJD case-based epidemiological model estimated prevalence of ~4.5 in 1,000,000 (Clarke and Ghani 2005), or a mean of approximately 1.23 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 ~4.5 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	*0.0707* (0– 1)[b]	*0.0203* (0– 0)[b]	*4.66* (0– 11)	*1.39* (0 – 4)
Number vCJD donors NOT DEFERRED (ineffective screening)	*0.0050* (0– 0)[b]	*0.00005* (0– 0)[b]	*0.34* (0-2)	*0.003* (0– 0)[b]
Number vCJD donors NOT DEFERRED (short-term travel <3 mos, UK; <5 yrs, FR and EU)	*0.0069* (0– 0)[b]	0.0067 (0– 0)[b]	*0.43* (0 – 2)	0.46 (0–2)[b]
Total number vCJD infected donors NOT DEFERRED Donate to pdFVIII Plasma Pools	***0.0186*** *(0 – 0)[b]*		***1.23*** *(0 – 4)*	

[a] Risk estimates generated by the model should fall within the interval defined by the 5th- 95th perc (percentiles) 90% of the time.

[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 95% 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 95% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

IV. C. Estimation of Annual Number and Percentage of Plasma Pools Potentially Containing vCJD Agent

The annual number and percentages of source or recovered plasma pools, potentially containing vCJD agent used to manufacture pdFVIII in the US were estimated by the model. The starting material for manufacturing pdFVIII is a plasma pool containing donations from thousands of donors. In this section, model first estimates the probability that an infected plasma pool is present. 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 majority of pdFVIII is manufactured from Source Plasma and the minority from recovered plasma. Source Plasma pools are usually smaller than recovered plasma pools. 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 they might contribute multiple donations to a single plasma pool or donate to multiple pools.

The probability that an infected plasma pool occurs is calculated using a binomial distribution probability function with parameter n equal to number of donors per plasma pool and parameter p equal to the vCJD prevalence of plasma. The model estimates that the probability an infected plasma pool containing plasma from multiple infected donors is small (at the least 2 magnitudes lower than the probability an infected pool containing plasma from only one infected donor). Therefore, the model assumes only one infected donors may be present in a plasma pool, if present at all. The model assumes number of infected plasma pools is equal to the number of donors who are infected and with infectious agent in blood at the time of donation.

The model also used market data for pdFVIII product, combined with estimated yield of pdFVIII per liter plasma and information on the number of donations per pool by type (either source or recovered) to calculate annual volume of plasma and annual number of plasma pools used to produce pdFVIII products distributed in the US in 2002.

Results-Module 2: vCJD risk of US plasma pools

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 Case Prevalence estimate of ~4.5 in 1,000,000 (based on Clarke and Ghani, 2005). The lower prevalence estimate used in the FDA model suggested that an average of 0.03% of all US plasma pools used to manufacture pdFVIII in the year 2002 potentially contained the vCJD agent (**Table 4.5**). In fact, on average >95% of the time plasma pools would be predicted not to contain a donation from a vCJD infected donor. Only an average of 0.10% recovered plasma pools are 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 5th and 95th percentile values of 0); suggesting that at least 95% of the time zero pools would contain the agent. Also at the lower prevalence, a vCJD donation in a Source Plasma pool would be predicted to be even more infrequent. Given the relatively small number of pools used annually in the United States (mean of 63) to produce pdFVIII the model predicts a positive pool to occur on average at a rate of 1 in 53 years.

HIGHER UK vCJD Infection 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 2.30% 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, 7.10% of recovered pools and 1.30% 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 of ~4.5 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	0.02% (0 – 0%)[b]	0.10% (0 – 0%)[b]	1.30% (0 – 5.60%)	7.10% (0 – 29.0%)
Mean percentage of pools potentially containing vCJD agent	**0.03%** (0 – 0%)[b]		**2.3 %** (0 – 8.2%)	

[a] Risk estimates generated by the model should fall within the interval defined by the 5^{th}- 95^{th} perc (percentiles) 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 95% 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. Estimation of the Quantity of vCJD agent in a Plasma Pool that Contains a Donation from a Donor Infected with vCJD

This section of the risk assessment estimates the quantity of vCJD agent in each vCJD plasma donation and pool that may be used to make pdFVIII. The quantity of infectious agent present in plasma pools may vary depending on the infectivity of each infected donation and the number of infected donations in the pool.

IV.D.1. Quantity of vCJD Agent Present in a Donation of a Donor Infected with vCJD

Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. 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 (5th percentile, most likely, and 95th 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. 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).

Assumption used in the model: *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 medium 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.

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 0.1 and 1.

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. The FDA risk assessment used a uniform distribution (0.1, 1) to represent of efficiency of transmission through i.v. route versus i.c. 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.D. 2. Quantity of vCJD Agent in a Plasma Pool Containing a Donation from Donor Infected with vCJD

The quantity of vCJD agent 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 present in a plasma pool 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. the prevalence of vCJD in the US is very low and the chance a pool involves multiple donations from vCJD infected donors is small.

Assumption used in the model: We assumed an average number of donations that individual donor would contribute to a Source plasma pool is -(b)(4)- units. The model used a Pert distribution with a minimum of -(b)4), most likely of -(b)(4), and maximum of -(b)(4)- to represent the uncertainty for this estimate. Individual infected recovered plasma donors most likely give only one donation to a pool.

MODULE 3 (IV.E)— CLEARANCE OF vCJD INFECTIVITY DURING MANUFACTURE OF pdFVIII

IV.E. Clearance of vCJD Infectivity 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. 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, fractionation and purification of individual protein component such as FVIII may partially remove TSE infectivity in the protein component. The fractionation and purification steps include alcohol precipitation, size exclusion, affinity chromatography, etc., 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. 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.

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	1-3	(Farrugia 2002; Lee *et al* 2002)
3% PEG	2-4	(Farrugia 2002)
11.5% PEG	1.7	(Foster *et al* 2000)
$Zn+Al(OH)_3$	2.7-3.5	(Foster *et al* 2000; Cervenakova *et al* 2002)
Ion exchange	1	(Foster *et al* 2000)
Membrane filtration		
Immunopurification	4.4-6.3	(Foster, Welch *et al* 2000; Cervenakova *et al* 2002)

IV.E. 1. Estimated Quantity of vCJD Agent per IU FVIII Product made from a Specific vCJD Plasma Pool

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 quantity of vCJD agent in FVIII product made from different infected plasma pool may vary depending on the initial quantity of vCJD agent in the plasma pool, the infectivity clearance through manufacturing, and the yield of FVIII product per liter of plasma. Considering the uncertainty in the degree of clearance that can be achieved during various pdFVIII manufacturing processes, this risk assessment models two levels of potential clearance in infectivity, 4-6 $\log_{10}$ and 7-9 $\log_{10}$.

<u>Assumption used in the model</u>: yield of FVIII (including high purity and intermediated purity FVIII) most likely is 150 IU (International Unit) per liter plasma with minimum of 130 and

maximum of 270 IU per liter plasma, and represented by a pert distribution (130, 150, 270) (WFH, 2004).

IV.E. 2. Estimated Percentage of FVIII Vials that Contain vCJD Agent

This section calculated the percentage of FVIII vials that contain vCJD agent. The percentage of FVIII vials that contain vCJD agent is used in the later section of the model to determine the likelihood of an individual patient receiving vCJD vial(s) basing on amount of FVIII product used by an individual patient.

Assumption used in the model: the percentage of FVIII vials that contain vCJD agent is equal to the percentage of FVIII plasma pools that contain vCJD agent, which is calculated in section IV. C.

Results-module 3: Per Vial vCJD Infection Risk for US Manufactured pdFVIII

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 ~4.5 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 0.03%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. ID_{50} per vial is 9.5×10^{-6} for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 210,000 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 700 million. Alternatively, this could be taken to mean that a patient would need to infuse 700 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. Readers may notice that the 5thand 95thpercentile intervals for all of the model

outputs using the lower prevalence estimate (~4.5 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 95% of the time 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, 0.03% of the time the exposure to vCJD may be greater than zero. Results indicate that using FVIII made from recovered plasma is likely have greater risk than using FVIII made from source plasma because a pool of recovered plasma contains plasma from more donors.

Per vial vCJD risk: Results based on the Higher vCJD Infection 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 2.3%. Assuming a clearance of 4-6 $\log_{10}$ the model estimates that the average quantity of i.v. ID_{50} per vial is 9.2×10^{-6} for vials produced from a contaminated pool. This result can be interpreted to mean that only 1 in 217,000 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 9.4 million. Alternatively, this could be taken to mean that a patient would need to infuse 9.4 million vials of product before accumulating one full infectious dose of vCJD. Similar to the results based on the Lower vCJD Case Prevalence estimate these results indicate that using FVIII made from recovered plasma is likely have greater risk than using FVIII made from source plasma because a pool of recovered plasma contains plasma from more donors.

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 Factor (LRF)					
	Model Output for LOWER vCJD Case Prevalence of ~4.5 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 vCJD risk[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 vCJD risk[b] (5^{th}- 95^{th} perc)[c]
Source	0.02% (0 – 0%)[d]	1.81×10^{-5} (6.65×10^{-7} – 6.89×10^{-5})	1 in 552 million (0, 0)[d]	1.30% (0 – 5.60%)	1.84×10^{-5} (8.26×10^{-7} – 6.73×10^{-5})	1 in 8.4 million (0, 1 in 531,000)
Recovered	0.10% (0 – 0%)[d]	1.55×10^{-6} (6.70×10^{-8} – 5.70×10^{-6})	1 in 1.3 billion (0, 0)[d]	7.10% (0 – 29%)	1.56×10^{-6} (8.65×10^{-8} – 5.42×10^{-6})	1 in 18.1 million (0,1 in 1.3 million)
Average of all vials	0.03 % (0 – 0%)[d]	9.50×10^{-6} (1.14×10^{-7} – 4.27×10^{-5})	1 in 620 million (0, 0)[d]	2.30% (0 – 8.2%)	9.23×10^{-6} (1.76×10^{-7} – 3.72×10^{-5})	1 in 9.4 million (0, 1 in 656,000)

*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] Risk estimates generated by the model should fall within the interval defined by the 5^{th} - 95^{th} perc (percentiles) 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 95% 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 95% of pdFVIII product lots (of vials) would not be predicted to contain vCJD agent.

Module 4 (IV. F): FVIII Utilization and Annual Exposure

FDA estimated that there are approximately 1,800 patients with severe hemophilia A (HA) disease and an estimated 250 patients with severe vWD in the US who use pdFVIII to manage their disease. Traditionally, HA patients were treated with factor concentrates only when bleeding

occurred, which is called episodic treatment. Patients are also treated using prophylactic therapy regimens that seek to prevent bleeding events through regular infusions of pdFVIII. Patients who need vWF must use plasma-derived sources of FVIII which contain vWF. No recombinant vWF is currently available.

IV. F. pdFVIII Utilization by HA and vWD Patients and Potential Exposure to the vCJD Agent

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
- probability of receiving infected pdFVIII product, and
- potential quantity of vCJD agent predicted in infected pdFVIII product.

IV. F. 1. Estimation of Annual Number vCJD Vials used by Individual Patient

The more a patient uses pdFVIII product the greater the chance they may receive an infected product. The quantity of pdFVIII utilized by an individual patient is dependent on the severity of the disease and the treatment regimen.

IV. F. 1. a. Estimation of pdFVIII Utilization by Patients with Severe Hemophilia A Disease

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 and Prevention (CDC) sponsored study in 6 states 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 and patient subpopulation:

- 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 is provided in the table below. We 'fit' different parametric models to the actual patient product utilization data and chose the model which best approximated the overall pattern of product use by each specific patient subpopulation. The beta distribution provided the best fit to the utilization data for each patient subpopulation, and therefore, was chosen as the input distribution for the variable of "FVIII annual usage". Each distribution was

truncated by minimum and maximum FVIII usage for each of the patient clinical treatment subpopulations.

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	5^{th}, 95^{th} percentiles
Prophylaxis	No Inhibitor	578	(300, 1200000)	157949	(21000, 382,000)
	With Inhibitor – No Immune Tolerance	63	(2000, 1000000	190523	(27000, 448000)
	With Inhibitor – With Immune Tolerance	62	(10000, 4000000)	558700	(33000, 1593000)
Episodic	No Inhibitor	946	(0, 1000000)	85270	(4600, 245000)
	With Inhibitor	151	(2000, 1000000)	160458	(5000, 489000)

IV. F. 1. a. FVIII Utilization in Patients with Severe von Willebrand disease

The CDC 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 indicate 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	5th, 95th percentiles
Young (≤15 yrs of age)				
Prophylaxis	9	(9200, 504625)	165713	(9900, 454000)
Episodic	14	(1010, 41850)	11045	(1020, 34350)
Adult (>15 yrs of age)				
Prophylaxis	17	(15000, 772800)	186880	(17000, 540000)
Episodic	18	(1000, 293800)	86923	(2200, 240000)

IV. F. 2. Quantity of vCJD Agent in pdFVIII Vials

This section of the model randomly draws vCJD vials received by individual patient from different plasma pools. The amount of infectious agent in vCJD vials vary because of variation on initial

infectivity of plasma pool, degree of infectivity clearance in manufacture processing and yield of product.

Assumption used in the model: The pdFVIII vials a patient received are randomly drawn from different plasma pools

IV. F. 3. Estimation of Potential Annual Exposure

This section of the model calculates the final output of the model, the annual predicted exposure of individual patient (ID_{50} per person, year). It is the total amount of infectious agents from all vCJD vials received by the patent during a one year period.

Assumption used in the model: *Exposure to vCJD is accumulative during one year period*

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 (dose-response) for humans 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 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 up to 1 million 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 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. We used visual graphic methods to verify that model estimates converged.

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 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 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 the 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 ~4.5 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) have 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 1.80×10^{-6} i.v. ID_{50} per person per year; representing an average potential vCJD risk of 1 in 1.1 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 18,000 years of treatment among severe HA patients who are in a prophylaxis treatment regimen with inhibitor and immune tolerance. As mentioned earlier the 5^{th} and 95^{th} percentile intervals for all of the model outputs using the lower prevalence estimate (~4.5 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 95% of the time the model estimated the risk to be zero because vCJD agent was not present in pdFVIII product used during treatment. However, the model predicted that 0.03% 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 was 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 7.79×10^{-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 2,600 years. At this expected level of risk, 1 vCJD infection would be predicted to occur in 2,600 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 1.10×10^{-4} i.v. ID_{50} per person per year, using the higher prevalence estimate. This represents an average potential vCJD risk of 1 in 18,000 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 290 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 4.9×10^{-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 41, i.e., 1 vCJD infection would be predicted to occur in 41 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 Factor (LRF)			
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		Model Output for HIGHER vCJD Infection Prevalence 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)	Mean exposure to vCJD iv ID_{50}[a] per person per year (5th - 95th perc)[c]	Mean potential vCJD risk per person per year[b] (5th - 95th perc)[c]	Mean exposure to vCJD iv ID_{50}[a] per person per year (5th - 95th perc)[c]	Mean potential vCJD risk per person per year[b] (5th - 95th perc)[c]
Prophylaxis	No Inhibitor	578	157,949 IU#[d] (21,000, 382,000)	5.10×10^{-7} (0-0)[e]	1 in 4.0 million (0-0)[e]	3.2×10^{-5} ($0-1.50\times10^{-4}$)	1 in 63,000 (0 - 1 in 13,000)
	With Inhibitor – No Immune Tolerance	63	190,523 IU#[d] (27,000, 448,000)	5.90×10^{-7} (0-0)[e]	1 in 3.4 million (0-0)[e]	3.90×10^{-5} ($0-1.90\times10^{-4}$)	1 in 53,000 (0 - 1 in 11,000)
	With Inhibitor – With Immune Tolerance	62	558,700 IU#[d] (33,000, 1,593,000)	1.80×10^{-6} (0-0)[e]	1 in 1.1 million (0-0)[e]	1.10×10^{-4} ($0-5.40\times10^{-4}$)	1 in 18,000 (0 - 1 in 3,700)
Episodic	No Inhibitor	946	85,270 IU#[d] (46,000, 245,000)	2.80×10^{-7} (0-0)	1 in 7.1 million (0-0)[e]	1.70×10^{-5} ($0-8.20\times10^{-5}$)	1 in 115,000 (0 - 1 in 24,000)
	With Inhibitor	151	160,458 IU#[d] (5,000, 489,000)	5.00×10^{-7} (0-0)[e]	1 in 4.0 million (0-0)[e]	3.30×10^{-5} ($0-1.50\times10^{-4}$)	1 in 61,000 (0 - 1 in 13,000)

[#] Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[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] Risk estimates generated by the model should fall within the interval defined by the 5th - 95th perc (percentiles) 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 95% 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 Factor (LRF)			
			Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		Model Output for HIGHER vCJD Infection Prevalence of 1 in 4,225 by Hilton *et al* (2004)	
	Est. Total Number severe HA patients in US	Mean quantity product used per person per year	Mean exposure to vCJD iv ID_{50} [a] per person per year (5^{th} - 95^{th} perc) [c]	Mean potential vCJD risk per person [b] per year (5^{th} - 95^{th} perc) [c]	Mean exposure to vCJD iv ID_{50} [a] per person per year [c] (5^{th} - 95^{th} perc)	Mean potential vCJD risk per person [b] per year (5^{th} - 95^{th} perc) [c]
Mean total annual exposure and population risk	1,800	243 million IU [d]	7.79×10^{-4} (0-0) [e]	1 in 2,600 years (0-0) [e]	4.90×10^{-2} ($0 - 2.39 \times 10^{-1}$)	1 in 41 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] Risk estimates generated by the model should fall within the interval defined by the 5^{th} - 95^{th} perc (percentiles) 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 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 95% 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 95% 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 Log_{10} Reduction Factor (LRF)			
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		Model Output for HIGHER vCJD Infection Prevalence 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] (9900, 454300)	5.10×10^{-7} (0 - 0)[e]	1 in 3.8 million (0 - 0)[e]	3.40×10^{-5} ($0 - 1.60\times10^{-4}$)	1 in 59,000 (0 - 1 in 12,000)
	Episodic	60	11,045 IU#[d] (1020, 34350)	3.60×10^{-8} (0 - 0)[e]	1 in 56 million (0 - 0)[e]	2.30×10^{-6} ($0 - 9.50\times10^{-6}$)	1 in 830,000 (0 - 1 in 210,000)
ADULT vWD (> 15 yrs of age)	*Prophylaxis*	73	186,880 IU#[d] (17000, 540000)	5.80×10^{-7} (0 - 0)[e]	1 in 3.4 million (0 - 0)[e]	3.80×10^{-5} ($0 - 1.80\times10^{-4}$)	1 in 53,000 (0 - 1 in 11,000)
	Episodic	78	86,923 IU#[d] (2200, 240000)	2.70×10^{-7} (0 - 0)[e]	1 in 7.1 million (0 - 0)[e]	1.80×10^{-5} ($0 - 8.50\times10^{-5}$)	1 in 114,000 (0 - 1 in 23,000)

[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.

#Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[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] Risk estimates generated by the model should fall within the interval defined by the 5^{th} - 95^{th} perc (percentiles) for 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 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 95% 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 95% 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 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 Factor (LRF)			
			Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)		Model Output for HIGHER vCJD Infection Prevalence 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	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	27.5 million IU[#][d]	8.60×10^{-5} (0 - 0)[e]	1 in 23,000 years (0 - 0)[e]	5.59×10^{-3} ($0 - 2.72\times10^{-2}$)	1 in 358 years (0 - 1 in 74)

[#] Mean Total quantity FVIII used by all patients was incorrectly reported as 29.9 million IU in the FDA 2006 risk assessment, the value of 27.5 million IU is the correct value

[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] Risk estimates generated by the model should fall within the interval defined by the 5th - 95th perc (percentiles) 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 95% 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 95% 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 ~4.5 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 5.80×10^{-7} i.v. ID_{50} per person per year, and representing an average potential vCJD risk of 1 in 3.4 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 46,600 years. As mentioned earlier the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate (~4.5 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 95% of the time 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 0.03% 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 27.5 million IU, and are potentially exposed to an average total of 8.60×10^{-5} i.v. ID_{50} per year. This represents an average potential vCJD risk of 1 in 23,000 **(Table 5.2B.)** or (as predicted by the model) roughly equal to one vCJD infection observed over a time span of approximately 23,000 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 3.80×10^{-5} i.v. ID_{50} per person per year, representing an average potential vCJD risk of 1 in 53,000 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 721 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 27.5 million IU **(Table 5.2B.)**, and are potentially exposed to an average total of 5.59×10^{-3} i.v. ID_{50} per year. This represents an average potential vCJD risk of one vCJD infection observed over a time span of 358 years for the population of 250 severe vWD patients in the U.S.

Range of Predicted Annual Mean Potential vCJD risk per HA patient 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 4.5 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 60 fold.

The model evaluated two separate categories of reduction in infectivity including 4-6 $\log_{10}$, and 7-9 $\log_{10}$. These two 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 two levels of reduction. 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 60,000 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 *two levels of clearance: 7-9 log_{10} and 4-6 log_{10}, at Higher Prevalence and Lower Prevalence estimates*

				7 - 9 Log_{10} Reduction Factor (LRF)		4 - 6 Log_{10} Reduction Factor (LRF)	
				Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence of 1 in 4,225 by Hilton *et al* (2004)	Model Output for LOWER vCJD Case Prevalence ~4.5 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence 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 (5th - 95th)[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]	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	157,949 IU[d] (21,000 , 382,000)	1 in 4.0 billion (0-0)[e]	1 in 63 million (0 - 1 in 13 million)	1 in4.0 million (0-0) e	1 in 63,000 (0- 1 in 13,000)
	With Inhibitor – No Immune Tolerance	63	190,523 IU[d] (27,000, 448,000)	1 in 3.3 billion (0-0)[e]	1 in 53 million (0 - 1 in 11 million)	1 in3.4 million (0-0) e	1 in 53,000 (0- 1 in 11,000)
	With Inhibitor – With Immune Tolerance	62	558,700 IU[d] (33,000, 1,593,000)	1 in 1.1 billion (0-0)[e]	1 in 18 million (0 - 1 in 3.7 million)	1 in1.1 million (0-0) e	1 in 18,000 (0- 1 in 3,700)
Episodic	No Inhibitor	946	85,270 IU[d] (46,000, 245,000)	1 in 7.1 billion (0-0)[e]	1 in 115 million (0 - 1 in 24 million)	1 in7.1 million (0-0) e	1 in 115,000 (0- 1 in 24,000)
	With Inhibitor	151	160,458 IU[d] (5,000, 489,000)	1 in 3.8 billion (0-0)[e]	1 in 63 million (0 - 1 in 13 million)	1 in4.0 million (0-0) e	1 in 61,000 (0- 1 in 13,000)

[a] Some numbers on quantity of product used that also appear in the 2006 FDA Risk Assessment have been rounded for simplification in the 2010 Updated FDA Risk Assessment

[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 iv ID_{50} per year x 0.5 (50 % chance infection from ID_{50})

[d] Risk estimates generated by the model should fall within the interval defined by the 5^{th} - 95^{th} perc (percentiles) 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 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 95% 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.

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This range or difference in the estimates of about 10 -20 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 1.1 billion. 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 7.1 billion.

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 4.5 in 1,000,000 and 7-9 $\log_{10}$ reduction, and
- Higher Prevalence assumptions of Prevalence of 1 in 4,225 and 4-6 $\log_{10}$ reduction.

			7 - 9 $\log_{10}$ Reduction Factor (LRF)		4 - 6 $\log_{10}$ Reduction Factor (LRF)	
			Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence of 1 in 4,225 by Hilton et al (2004)	Model Output for LOWER vCJD Case Prevalence of ~4.5 in 1,000,000 based on Clark and Ghani (2005)	Model Output for HIGHER vCJD Infection Prevalence 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	Mean population – based potential vCJD risk[a] (5th - 95th perc)[c]	Mean population – based potential vCJD risk[a] (5th - 95th perc)[c]	Mean population – based potential vCJD risk[a] (5th - 95th perc)[c]	Mean population – based potential vCJD risk[a] (5th - 95th perc)[c]
Mean total annual exposure and population risk	1,800	243 million IU	1 vCJD infection in 2.6 million years (0-0)[c]	1 vCJD infection in 40,000 years (0 -1 in 8,000)	1 vCJD infection in 2,600 years (0 -0)[c]	1 vCJD infection in 41 years (0 -1 in 8)

[a] Mean potential annual vCJD risk – the risk of potential vCJD infection based on animal model dose-response information.

[b] Risk estimates generated by the model should fall within the interval defined by the 5th - 95th perc (percentiles) 90% of the time.

[c] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 95% 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 95% 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 infection in 2.6 million years representing a negligible vCJD risk that would likely not give rise to new cases of the disease.

V. D. Sensitivity Analysis

A sensitivity analysis was conducted to determine which inputs in the model would have the largest impact on estimates of exposure to ID50s of the agent responsible for causing vCJD. The sensitivity analysis used the exposure of an adult hemophilia patient who uses FVIII for prophylactic treatment and has developed immunity and inhibitors as the baseline. The baseline also assumed an average log reduction during processing of 4-7 logs. From this baseline, eight inputs—Efficiency of Deferral, Yield, IC to IV conversion, Prevalence in UK, IBL, Donors per Pool, Usage, and Log Reduction—were sequentially set to a constant low or high value while the rest of the model was unchanged. The low and high values were either the minimum and maximum or the 5th and 95th percentile (See Table 5.4). The sensitivity test was run using both the clinical and tissue prevalence estimates. For both the clinical and tissue models, the sensitivity test for the prevalence in the UK ranged from the 5th percentile of the clinical prevalence estimate to the 95th percentile of the tissue prevalence estimate.

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: 0.62 Maximum: 1,123
Efficiency of donor deferral policy	Eff_{Def}	Minimum: 85% Maximum: 99%
Efficiency of i.c. versus i.v. route	$A_{ic\text{-}iv}$	Minimum: 0.1 Maximum: 1
Number of donors per plasma pool	DR_{Pool}	Minimum: 6500 Maximum: 360000
Quantity of i.c. infectivity in infected human blood	I_{bl}	5th perc: 2 95th perc: 30
Manufacturing yield of FVIII (IU/L plasma)	Y_{VIII}	Minimum: 130 Maximum: 270
Log Manufacture Reduction of vCJD agent	R_{Log}	Minimum: 2 Maximum: 9
FVIII used per year (IU/year)	IU_{yr}	5th perc: 10000 95th perc: 4000000

The results of the sensitivity analysis are shown in tornado graphs (**Figure 2. A. and 2. B.**). The tornado graphs are centered on the mean exposure estimates for the clinical and tissue prevalence scenarios. The inputs are ordered from top to bottom based on the size of the absolute difference between the estimated mean exposure when the input was set to a high value compared to a low value. Bars extend from the overall mean exposure estimate to the estimates when the input was held constant at a low or high value.

For both the higher tissue (Figure 2.A.) and lower clinical (Figure 2.B.) prevalence scenarios, the log reduction during manufacturing had the largest impact on the estimate of exposure. In the clinical scenario using the Lower vCJD Case Prevalence estimate, the mean estimated exposure with log reduction during manufacturing set to 2 and is 1.2×10^{-3} compared to the baseline estimate of 1.7×10^{-6}. In the tissue prevalence scenario using the Higher vCJD Infection Prevalence estimate, the mean estimated exposure with log reduction during manufacturing set to 2 is 6.9×10^{-2} compared to the baseline estimate of 1.0×10^{-4}. Prevalence in the UK has the second highest impact on estimated exposure in the clinical prevalence scenario. The rest of the inputs in the clinical (Lower vCJD Prevalence) prevalence scenario have a relatively small impact on the estimated prevalence. In the tissue (Higher vCJD Prevalence) prevalence scenario, usage of FVIII had the second largest impact on the estimate of overall exposure followed by the number of donors per pool. The remaining inputs had relatively small impacts on the exposure estimate.

Figure 2.A.

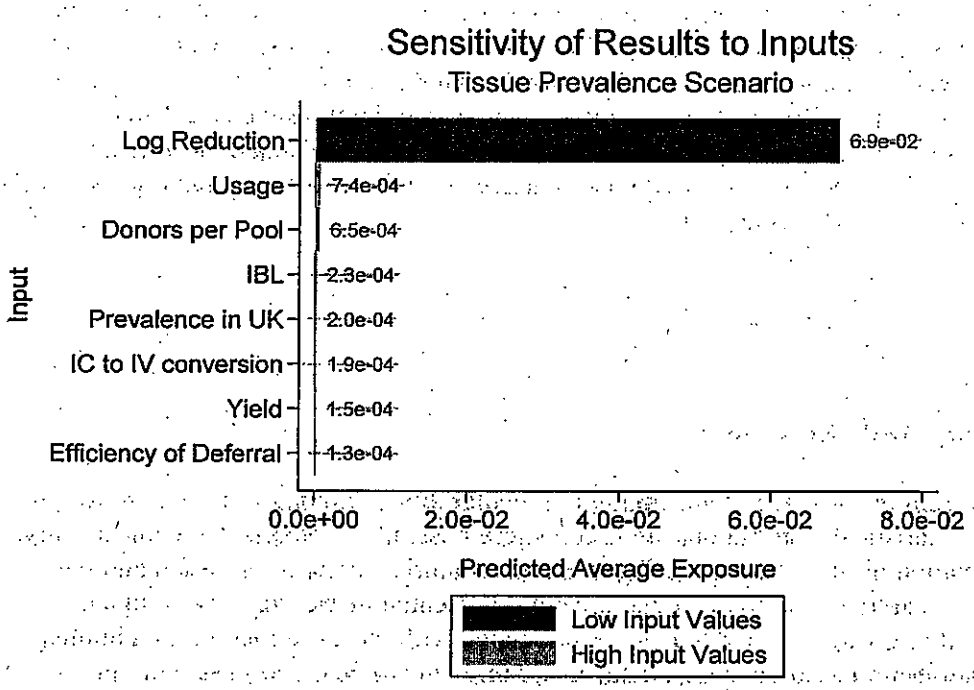
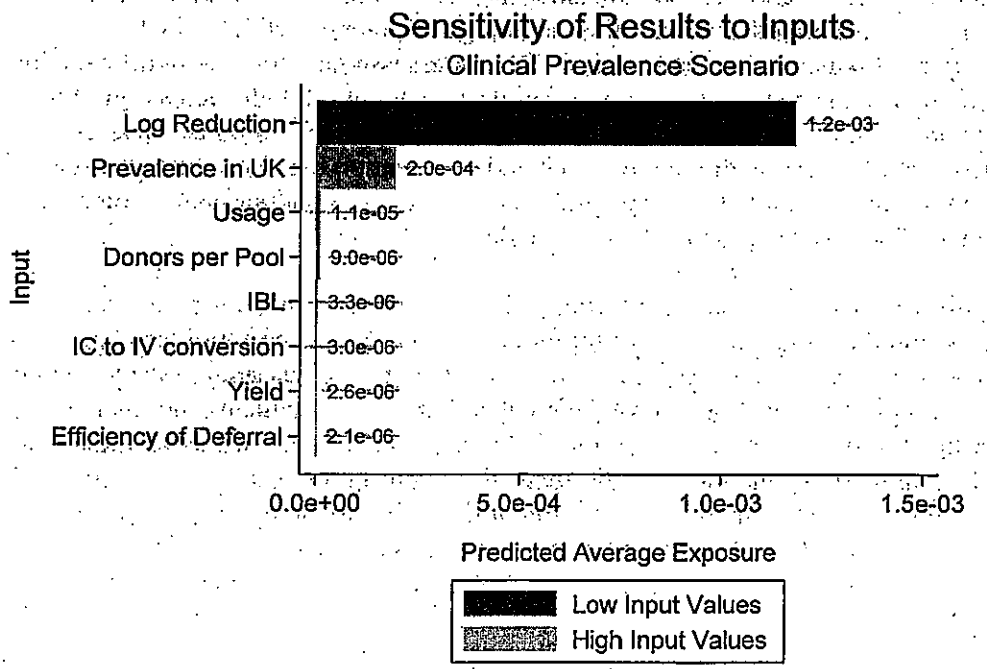


Figure 2.B.



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 of a statistical distribution representing the probabilities and range of potential vCJD risk. 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.D. 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 two ranges of possible clearance of vCJD agent from pdFVIII of 4-6 $\log_{10}$, and 7-9 $\log_{10}$ to cover the possible ranges for all pdFVIII products presently in the marketplace.

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 10,000 for the higher vCJD prevalence estimate and high product usage to 1 in 4.0 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, the amount of product that individuals used, and the vCJD prevalence in the UK donor population.

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APPENDIX A

Supplemental technical information for the FDA Risk Assessment

Appendix A provides additional technical information of modeling approaches and details of data 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 document and the model spread sheet. The model was developed using @Risk computer software version 5.5 (Palisade Co.).

Most of the data and information used in the model were converted to statistical distributions, whenever possible, representing the variability and uncertainty associated with the input variables. In general, we used a single value, or point estimate, if no information was available that could be used to quantify the variability and uncertainty. We used a uniform distribution, represented by a minimum and maximum value, when there was only enough information to define a range; a triangular distribution, represented by a minimum, most likely, and maximum value, when there was enough information to define a range and most likely value. Other more sophisticated parametric distributions were used when there sufficient data available so that we could evaluate, or 'fit', several possible distributions and choose the distribution that best reflected the pattern of the data. In other cases, we used point estimate for sets of correlated input variables such as donation rates by individual age group and percentage travel by destination. Applying statistical distributions to these variables would greatly complicate the model and expand the computational time required to generate model results by several days. We believe point estimates give a reasonable representation of the input variables. However, we do acknowledge that the use of point estimates may underestimate the uncertainty associated with the input variables.

Module 1. Prevalence of vCJD in the United Kingdom (including section A-IV. A.)

A- IV.A. Prevalence of vCJD in the United Kingdom (Module 1)

A-IV. A.1. UK asymptomatic vCJD infections estimated using epidemiological modeling results (Clarke and Ghani 2005) and adjusted for all three genotypes

A-IV.A.1.a. Estimation of the number asymptomatic vCJD infections in UK in 2002

Variable: $Asym\text{-}vCJD_{UK}$ - Number of asymptomatic vCJD infected individuals in the UK in year 2002.

See worksheet "Model-IV. Exposure Assessment" for full calculations.

Predicted vCJD clinical cases from 2002 onward include 32 vCJD cases diagnosed from 2002 to 2003 and 70 (95% CI of 10-190) cases predicted by Clarke and Ghani (2005) for years from 2004 to 2080. This gives a total of 102 predicted clinical cases (95% CI: 42-222 cases) from 2002 onward. Predicted total

vCJD clinical cases from 2002 onward represent the number of asymptomatic vCJD individuals in 2002 among *PRNP*-129-MM (MM) genotype, because, to date, all reported clinical vCJD cases have been come from MM genotype.

The number of asymptomatic vCJD individuals in 2002 among MM genotype was used in 2006 risk assessment for calculation of UK vCJD case prevalence. However, recent evidence suggested that all genotypes including persons who are Methionine-Valine (MV) heterozygous or Valine (VV) homozygous at codon 129 of PRNP are susceptible to the disease. Reports of *PRNP*-129-non-MM (non-MM) genotype individuals with immuno-histochemical evidence of vCJD infection detected post-mortem have been published in the literatures (Peden 2004, Ironside 2006). In 2009, FDA began updating the risk assessment. As part of that work and the 2010 update we multiplied the case prevalence estimate used in 2006 version of the risk assessment by a factor of 2.5, which expands the previous Lower vCJD Case Prevalence estimate based on only the MM population to include the MVand VV genotyped populations as well. The factor of 2.5 was derived from dividing 100% (representing whole population) by 40% (representing percentage of MM population). Therefore, the estimated total number of asymptomatic vCJD infections in overall UK population of all genotypes in 2002 has an average of 255 cases ((32 + 70) x 2.5 = 255) with a 5th percentile of 105 cases ((32+10) x 2.5 = 105) and 95th percentile of 555 cases ((32+190) x 2.5 = 555). This new estimate was used in 2010 update of the FDA risk assessment for calculation of vCJD case prevalence estimate for UK population.

A-IV. A. 1. b. Age distribution of asymptomatic vCJD for different genotypes

Cases of vCJD occur in relatively young individuals (median age of 26 years (Wadsworth and Collinge, 2007)) compared to classic CJD. Blood and plasma donors are usually range from 18 – 40 years of age, among whom the vCJD prevalence would be expected to be higher than that of the general population (Table A.1-1). 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. 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 throughout the FDA model in estimating vCJD risk and prevalence for the residents of different geographic regions (UK, France and other countries in Europe) and the US blood and plasma donors who traveled to those regions.

The 2006 version of FDA model used age information on reported vCJD clinical cases to calculate the age-specific percentage and prevalence of vCJD infections. The age specific percentage of reported vCJD cases is shown in **Table IV.A.1-1**. For a more accurate calculation the 2010 updated version of FDA model used age at the infection (shifting toward younger age to include incubation period) to calculate the age-specific percentage and prevalence of vCJD infections.

Table IV.A.1-1. 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	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

A-IV. A. 1. b. i. Percentages of PRNP genotypes in the population

Variable: $Perc_{MM}$, $Perc_{MV}$ and $Perc_{VV}$: Percentages of MM, MV and VV genotypes among the population.

Assumption used in the model: The model assumes 40%, 50% and 10% population are MM, MV and VV genotypes, respectively (Alpeovitch et al 1999), these values were used as point estimates in the model..

A-IV.A.1.b.ii. Percentage asymptomatic vCJD attributed to age groups and genotypes

Calculations for this section are in the model worksheet "age-asy-vCJD", and are described in detailed below:

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. Five reported cases (**Table IV.A.1-1.**) have been identified in the 55-74 yr age range (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)). We used an average of 1.25 cases for each of the four age groups, 55-59 yrs, 60-64 yrs, 65-69 yrs and 70-74 yrs to estimate the percentages for each group.

Variable: $Pr(infected)_{MM\text{-}age}$, $Pr(infected)_{MV\text{-}age}$, $Pr(infected)_{VV\text{-}age}$: Probability of infection at a specific age for a specific genotype.

The probability of infection was calculated as cumulative form of distribution of age at time of initial infection. The distribution of age at time of initial infection was calculated by left shifting the distribution of age at diagnosis for MM genotype, which was generated using age information on reported clinical cases, by a unit representing incubation period for MM genotype.

Assumption used in the model: Probability *PRNP*-129-MV and -VV genotypes having been infected at specific age are same as -MM genotype; and some of -MV and -VV will eventually develop overt disease, and their blood may contain the infectious vCJD agent for a portion of the incubation period.

Assumption used in the model: Three genotypes were equally susceptible to the disease, thus, the derived distribution of age at time of initial infection for MM genotype was also applied to other two genotypes.

Assumption used in the model: Based on information of BSE and vCJD epidemics, estimated incubation period for MM genotype ranges from 10 to 15 years.

The estimation of incubation periods for MV and VV genotypes remains complicated and more uncertain, because so far there have been no clinical cases reported from MV and VV genotypes. Given this considerable uncertainty, we made simplifying assumptions that the incubation period for MV and VV is 20 year longer than that for MM genotype. The 95th percentile values of incubation period for MV and VV are 55 years, which was estimated based on the maximum incubation period for kuru (Collinge 2006).

Assumption used in the model: The incubation period is presented by gamma distribution with median of 12 year (90%CI; 5-35 years) for *PRNP*-129-MM genotype and a median of 32 years (90%CI; 25-55 years) for non-MM genotypes.

Variable: $Pr(diagnosed)_{MM\text{-}age}$, $Pr(diagnosed)_{MV\text{-}age}$, $Pr(diagnosed)_{VV\text{-}age}$: Probability of being diagnosed at a specific age for a specific genotype given infection.

Probability of being diagnosed is calculated as cumulative form of distribution of age at time of diagnosis. The distribution of age at diagnosis for MM genotype is generated using age information on reported clinical cases. The distribution of age at time of diagnosis for MV and VV genotype is generated by right shifting distribution of age at time of diagnosis for MM by a unit representing extra incubation period needed for MV and VV compared to MM genotype.

Assumption used in the model: The extra incubation period needed for MV and VV compared to MM genotype is approximately 20 years.

Variable: $Pr(asym\text{-}vCJD)_{MM\text{-}age}$, $Pr(asym\text{-}vCJD)_{MV\text{-}age}$, $Pr(asym\text{-}vCJD)_{VV\text{-}age}$: Probability of asymptomatic vCJD infections for a specific age group and genotype.

The probability of asymptomatic vCJD infections is calculated by a joint probability of being infected and not being diagnosed. An example equation for MM genotype is shown below:

$$\Pr(asym-vCJD)_{MM-age} = \Pr(\text{inf}\,eced)_{MM-age} \times (1-(\Pr(diagnosed)_{MM-age}) \qquad \text{(IV.A.1-1)}$$

Variable: $Perc_{asy\text{-}vCJD(age)\text{-}MM}$, $Perc_{asy\text{-}vCJD(age)\text{-}MV}$, **and** $Perc_{asy\text{-}vCJD(age)\text{-}VV}$,: Percentage asymptomatic vCJD cases attributed to different age groups and genotypes.

The probability of asymptomatic vCJD infections were normalized so that all age groups of all genotypes added up to 1, then, multiplied by percentage of three genotype in the population to arrive percentages of asymptomatic vCJD cases attributed to different age groups and genotypes,

A-IV. A. 1. b. iii. Number of asymptomatic vCJD in a specific age group with a specific genotype

See worksheet "Model-IV. Exposure Assessment" for display of full calculations.

Variable: $Asy\text{-}vCJD\text{-}MM_{(age)}$, $Asy\text{-}vCJD\text{-}MV_{(age)}$, $Asy\text{-}vCJD\text{-}VV_{(age)}$ - Number of asymptomatic vCJD infected individuals from a specific age group in year 2002.

Variable: $Asym\text{-}vCJD_{UK}$ - Number of asymptomatic vCJD infected individuals in the UK in year 2002 (estimated in section IV-A.1.a)

$$Asym\text{-}vCJD\text{-}MM_{(age)} = Asym\text{-}vCJD_{UK} \times Perc_{asy\text{-}vCJD(age)\text{-}MM} \qquad \text{(IV.A.1-2)}$$

A-IV. A. 1. c. Prevalence of asymptomatic vCJD in the UK by age and genotype

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.

The prevalence of asymptomatic vCJD cases in the UK by age group and genotype is estimated using the equation:

$$Prev_{Asym\text{-}vCJD(age)\text{-}MM} = Asym\text{-}vCJD\text{-}MM_{(age)} / (Pop_{UK(age)} x Perc_{MM} \qquad \text{(IV.A.1-3)}$$

A-IV.A.2. UK asymptomatic vCJD infections derived from a tissue surveillance study (Hilton *et al* 2004)

This estimate was used in FDA 2006 risk assessment as UK vCJD infection prevalence, and remained same in the updated 2010 version of the FDA risk assessment.

A-IV.A.2.a. UK asymptomatic vCJD prevalence of 20-30 years age group

A retrospective tissue surveillance study (Hilton et al 2004) examined 12,674 tonsil and appendix tissue samples surgically removed from UK patients for the accumulation of vCJD infectious agent. The research found 3 samples positive. This data was converted to an average rate of vCJD in the UK population of 1 in 4,225 or prevalence of 237 cases per million (Hilton et al 2004). 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, and the 3 positive samples were also from this age group. Demographic information of reported vCJD cases (Table IV.A.1-1) 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 population derived from the surveillance study might, therefore, be over-represented by the 20-29 years age group. Therefore, we used tissue surveillance data to calculate the vCJD prevalence for 20-30 years age group (one of age categories used in the model) using 60% sample size as denominator. We used 3 positives out of 7604 tested samples from age groups of 20-30 years (60% x 12674=7604) to determine the most likely value (3/7604) and 95% confidence intervals (determined using statistic procedure BinomialXac in software STATXAC). Assuming the sensitivity and specificity of the testing method is 100%, we calculated a vCJD prevalence of approximately 400 cases per million for which we assumed a 95% CI of 100-1200 cases per million for 20-30 year old group.

Variable: $Prev_{Asym-vCJD(20-30)}$ Prevalence of asymptomatic vCJD infected individuals in the UK 20-30 year old age group (cases/million)

Assumption used in the model: The vCJD infectious agent is present in the blood of the individual when the accumulation of prion protein can be detected in lymphoreticular tissue.

Assumption used in the model: 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.

A-IV.A.2.b. UK vCJD prevalence in specific age group with specific genotype

We determined the proportional difference between the percentage asymptomatic vCJD cases from the 20-30 years age group and that from each of the other age groups (estimated in A-IV.A.1.b.ii) and multiplied the estimated asymptomatic vCJD cases among 20-30 year age group to arrive the number of asymptomatic vCJD cases for remaining age groups.

Variable: $Pop_{UK(age)}$- Population in the UK by age groups (Thousands).

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:

$$Asym\text{-}vCJD_{(20\text{-}30)} = Prev_{vCJD(20\text{-}30)} \times Pop_{UK(20\text{-}30)} \qquad \text{(IV.A.2-1)}$$

Variable: $Asym\text{-}vCJD\text{-}MM_{(age)}$, $Asym\text{-}vCJD\text{-}MV_{(age)}$ $Asym\text{-}vCJD\text{-}VV_{(age)}$- Number of asymptomatic vCJD infected individuals in the UK by age groups of specific genotype.

Assumptions used in the model: Three genotypes are equally susceptible to the disease; therefore, total infection cases for a specific age group are attributed to three genotypes in proportion to the population sizes of three genotypes.

As an example, the number of asymptomatic vCJD individuals in the UK of specific age group with a MM genotype was estimated using the following equation:

$$Asym\text{-}vCJD\text{-}MM_{(age)} = Asym\text{-}vCJD_{(20\text{-}30)} \times (Perc_{vCJD(age)}/Perc_{vCJD(20\text{-}30)}) \; x \; Perc_{MM} \qquad \text{(IV.A.2-2)}$$

Variable: $Prev_{Asym\text{—}vCJD\text{-}MM(age)}$, $Prev_{Asym\text{—}vCJD\text{-}MM(age)}$, $Prev_{Asym\text{—}vCJD\text{-}MM(age)}$: Prevalence of asymptomatic vCJD infected individuals in the UK for specific age group with a specific genotype (cases/million).

Calculation for the prevalence of asymptomatic vCJD cases in the UK specific age group with specific genotype is same as shown in section **IV.A. 1. c.**

Module 2. Potential vCJD risk for US plasma donors and plasma pools (including sections A-IV.B., A-IV.C. and A-IV.D.)

A-IV.B. Estimation of vCJD prevalence in US plasma donors and plasma pools

Based on data FDA received from several manufacturers we assumed that a plasma pool used to manufacture pdFVIII product in the US in the year 2002 consisted of 6,000 to 360,000 donations, and several donations in the pool likely came from the same donor. In this section of the model the estimated vCJD prevalence in UK population was used to generate variables and parameters for calculation of vCJD prevalence in US plasma donors and the potential number of vCJD donors or donations that might be present in a plasma pool.

A-IV. B. 1. Annual number of plasma donors (See worksheet "Model-IV. Exposure Assessment")

A-IV. B. 1. a. 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: Based on data FDA received from manufacturers 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 information on annual units of pdFVIII made from Source Plasma collected in the US and unit volume of Source Plasma was 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 assumed to be 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.b. 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 back calculation 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 Recovered Plasma collected in the US was collected from pdVIII manufacturers.

Variable: $DN_{Bl\_perc(age)}$ – 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 Table IV. A.1.-1).

Variable: $DN_{R(age)}$ – Annual units of recovered plasma used to make pdFVIII by donor age group

$$DN_{R(age)} = DN_R \times DN_{Bl-perc(age)} \qquad \text{(IV.B.1-2)}$$

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:

$$DR_{R(age)} = DN_{R(age)} \qquad \text{(IV.B.1-3)}$$

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:

$$DR_R = \sum_{age=18-19}^{70-74} DR_{R(age)} \qquad \text{(IV.B.1-4)}$$

Assumption used in the model: Minimum age for a qualified donor is 18 years.

A-IV.B.1.c. 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.

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.

A-IV.B.2. Annual number of plasma donors potentially infected and whose blood may contain vCJD infectivity

This section of the model estimated the annual number of US plasma donors potentially exposed to vCJD, and infected during travel or residence in BSE epidemic regions, and potential number of infected donors whose blood contains vCJD agent at the time of donation in year 2002. We modeled five potential sources of risk for plasma donors separately, travelers to the UK, travelers to France, travelers to the other countries in Europe, military personal who have been deployed to military bases in Europe and Euroblood recipients.

A-IV.B.2.a. US plasma donors with history of travel to the UK: Annual number potentially infected and whose blood may contain vCJD agent

This section estimated numbers of donors who have history of travel to the UK and numbers of donors who might have been infected during travel in UK and whose blood may contain vCJD agent in 2002. Detailed calculations are on the model worksheet "IV. B. 2. a Travel-UK". The results of calculations were drawn back to the model main worksheet "Model-IV. Exposure Assessment" for subsequent calculations of the model.

A-IV.B.2.a.i. US plasma donors with history of travel to the UK: Percentage of donors and duration of travel (see worksheet "IV. B. 2. a Travel-UK")

The risk of vCJD infection in US plasma donors is a function of the intensity of exposure to the BSE agent. FDA model assumed the intensity of exposure 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. 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. We assume that the likelihood that infected animals or products from BSE-infected animals in the UK entered the human food supply after 1996 was small. 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 (>=3 months) in the UK during 1980-1996, and to derive the frequencies for various durations of travel less than 5 years. The accumulated durations of travel of 3 months or more corresponds to the length of time in the current policy that defers donors from blood donation.

The travel survey data on blood donors has limitations because it may not exactly reflect the travel histories of Source Plasma donors. Some may argue that source plasma donors travel less frequently than their blood donor counterparts so use of data on blood donors may overestimate the risk. Unfortunately, to our knowledge there is no travel data available on Source Plasma donors. Therefore, we assumed that Source 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 risk assessment. Recovered Plasma is plasma that is separated or "recovered" from a unit of whole blood soon after the blood is collected. As expected, the characteristics of Recovered Plasma donors mirror those of whole blood donors.

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:

$$Perc_{BIDR-UKi/UK} = (Perc_{BIDR-UKi} / CumPerc_{BIDR-UK,i>1day-1month}) \times 100\% \qquad \text{(IV.B.2.a-1)}$$

A-IV.B.2.a.ii. 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 who traveled to the UK between 1980 and 1996 into age groups of five year increments (20 – 24yrs, 25 – 29 yrs, and so on). Because the minimum age of donation is 18 years of age, 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 number of donors, total number donors who traveled to the UK between 1980 and 1996, and the age specific odds ratio for travel.

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. This information was 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. 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.

Assumption used in the model: There was zero exposure to the BSE agent for donors who traveled to the UK prior to 1980 and after 1996.

A-IV.B.2.a.ii.(1). US Source Plasma donors with history of travel to the UK: Estimation of the annual number of source plasma donors who traveled to the UK in a specific year by age group

The model generates categories (or bins) for Source Plasma donors by year of travel, so estimates of the risk can be more accurate by incorporating the information about dynamic change of BSE epidemic in the UK.

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).

Assumption used in the model: US Source Plasma donors have similar travel patterns as 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:

$$V_{y/1996} = V_y / V_{1996} \qquad \text{(IV.B.2.a-2)}$$

Variable: DR_s (calculated in section A-IV.B.1.) - The annual number of source Plasma donors.

Variable: $DR_{s(Age)}$ (calculated in section A-IV.B.1.) - The annual number of source Plasma donors by age group.

Variable: $Perc_{DR\text{-}UK}$: Percentage of donors who have history of travel to the UK. This percentage was derived based on American Red Cross's Donor Travel Survey.

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

Variable: $DR_{S\text{-}UK(age)(1980\text{-}1996)}$ - 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.

The number of Source Plasma donors who traveled to the UK from 1980 to 1996 by age group is represented by:

$$DR_{S\text{-}UK(age)(1980\text{-}196)} = DR_S \times Perc_{DR\text{-}UK} \times (DR_{S(Age)} \times Odd_{T(age)}) / \sum_{Age=18\text{-}19}^{70\text{-}74} (DR_{S(Age)} \times Odd_{T(age)}) \qquad \text{(IV.B.2.a-3)}$$

Source Plasma donors with a history of travel to the UK among each age group ($DR_{S\text{-}UK(age)(1980\text{-}1996)}$) 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 exclude the probability of travel of young donors (18-22 year old in 2002) in early years of BSE epidemic when they were not born. For example, donors of 18 years of age in 2002 were born in 1985, therefore, had no chance of travel and exposure to the BSE agent prior to 1985; those 19 years of age in 2002 had no chance of travel prior to 1984, those 20 years of age in 2002 had no chance of travel prior to 1983, those 21 years of age in 2002 had no chance of travel prior to 1982, and those 22 years of age in 2002 had no chance of travel in 1980.

Variable: $DR_{S\text{-}UK(age),y}$ - the number of Source Plasma donors who traveled to the UK in year y by age group

Assumptions used in the model: The yearly travel trend for blood and plasma donors are the same as trend for the general US population

The number of US Source Plasma donors who have traveled to the UK in year y between 1980 - 1996 is represented by the equation:

$$DR_{S\text{-}UK(age)y} = DR_{S\text{-}UK(age)} \times V_{y/1996} / \sum_{y=1980}^{1996} V_{y/1996} \qquad \text{(IV.B.2.a-4)}$$

A-IV. B. 2. a. ii.(2). US Source Plasma donors with history of travel to the UK: Duration of travel by age group

This section of the model used the data on the number of Source Plasma donors who have traveled to the UK in a specific year and divided those individuals into sub-categories by durations of stay. The model used categories of duration of stay used in the ARC's report for Blood Donor Travel Survey (TSEAC, 2000).

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 in section A-IV. B. 2. a. i.).

Variable: D_i - The average duration of time for interval i (months) (same variable used above in section A-IV. B. 2. a. 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. B. 2. a. ii (1))

Variable: $Perc_{DR\text{-}UKi/UK}$- 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.B.2.a.i)

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:

$$DR_{S-UK(age)y,i} = DR_{S-UK(age)y} \times Perc_{DR-UKi/UK} \qquad \text{(IV.B.2.a-5)}$$

A-IV. B. 2. a. ii.(3). Number of US recovered plasma donors with a history of travel to the UK in a specific year from 1980 – 1996 by age group

Calculations in this section are similar to the calculation for Source Plasma donors shown in section A-IV.B.2.a.ii.(1).

A-IV. B. 2. a. ii. (4). US recovered plasma donors with history of travel to the UK: Duration of travel by age group

Calculations in this section are similar to the calculation for Source Plasma donors shown in section A-IV.B.2.a.ii.(2).

A-IV. B. 2. 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

The FDA model assigned residents of the UK for any five-year period or longer from 1980 through 1996 a relative risk value of 1 (the highest value) for vCJD risk, 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. This was determined based on FDA guidance (2002), considering the factors such as domestic UK beef consumption, the rate and number of vCJD cases, and indigenous BSE cases that may have occurred (TSEAC 2004).

A-IV.B.2.a.iii.(1). Accumulated 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 for each UK individual is 1. The vCJD risk value of 1 corresponds to the vCJD prevalence among the UK population estimated in section IV-A.

A-IV.B.2.a.iii.(2). 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 and 1996.

Presumably there were dramatic variations in the BSE exposure risk, and hence, the human vCJD infection risk that occurred from year to year between 1980 and 1996. 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. Therefore, the model calculated the proportional BSE risk per year (e.g., the BSE exposure risk in a given year compared to the total accumulated BSE risk in the period from 1980 to 1996) in order to incorporate the changing dynamics of the BSE epidemic since 1980 and to account for the difference exposure risk for the donors who traveled to the UK at the different years.

Variable: y – year of travel (same as variable used above in section A-IV.B 2.a.ii.) by US plasma donor to the UK between 1980 and 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 between 1980 and 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 infection 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 between 1980 and 1996 is represented by the equation:

$$R_{UKy} = R_{UK} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.B.2.a-9)}$$

A-IV.B.2.a.iii.(3). 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.

The FDA model update for 2010 assumed the potential vCJD risk for the US plasma donors who traveled to the UK was also a function of duration. The potential vCJD risk for the US plasma donors who 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.

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.

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 vCJD risk for US plasma donors with a stay less than or equal to one year – is represented by the equation:

$$R_{DR-UKy,i} = (R_{UKy}/12) \times D_i \qquad \text{(IV.B.2.a-10)}$$

for i<=1 years;

The vCJD 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:

$$R_{DR-UKy,i} = (Average(R_{UKy} : R_{UK(y+Roundup(i_{upper,0}))}/12) \times D_i \qquad \text{(IV.B.2.a-11)}$$

for 5 years > $i \geq$ 1 year;

The vCJD risk for US plasma donors with a stay greater than or equal to five years – is represented by the equation:

$$R_{DR-UKy,i} = 1 \qquad \text{(IV.B.2.a-12)}$$

for $i \geq$ 5 years

A-IV.B.2.a.iv. US plasma donors with a history of travel to the UK: Probability of infection with vCJD based on duration of travel, age and genotype

This section describes the portion of the model that estimates the probability that a US plasma donor in a specific age group of a specific genotype, who traveled to the UK for a specific duration in a specific year during the time-span of 1980 through 1996, was infected with vCJD. In this revised risk assessment we incorporate the effect of all three donor genotypes for codon 129 of PNRP on the probability of infection, which was not considered in 2006 risk assessment. The vCJD prevalence for the UK population of specific age and genotype are calculated in risk assessment section A-IV.A. It represents the probability of infection for an average UK resident, who have value of 1 for vCJD risk. The probability of infection for an US plasma donor was adjusted for the year of travel and duration of the stay using vCJD risk associated with year of travel and duration of the stay calculated in section A-IV.B.2.a.iii. So far, there has been no sufficient information differentiating the susceptibility of MV and VV genotypes. In the updated 2010 version of the FDA model, MV and VV genotypes are modeled separately, but use same input values. Calculations for three genotypes are similar. Calculations for MM genotype are described below as an example.

Variable: $Pr_{Asym\text{-}vCJD\text{-}UK\text{-}MM(age)}$ – the probability of vCJD infection per individual UK resident of a specific age group for persons with the MM genotype

Variable: $Prev_{Asym\text{-}vCJD\text{-}MM(age)}$- Prevalence of asymptomatic vCJD infection in the UK MM population 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.).

$$\Pr_{asym\text{-}vCJD\text{-}UK\text{-}MM(age)} = \Pr ev_{Asym\text{-}vCJD\text{-}mm(age)} \quad \text{(IV.B.2.a-13)}$$

Variable: $Pr_{vCJD\text{-}MM\text{-}DR\text{-}UK(age)y,i}$ – The probability of infection for individual US plasma donor of a specific age group with the MM genotype 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

$$\Pr_{vCJD\text{-}MM\text{-}DR\text{-}UK(age)y,i} = \Pr_{Asym\text{-}vCJD\text{-}UK(age)} \times R_{DR\text{-}UKy,i}$$

(IV.B.2.a-14)

Calculations for MV and VV genotypes are similar to those for MM genotype and are not repeated here.

A-IV. B. 2. 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. The model estimates the number of potentially infected Source and recovered plasma donors for each of three genotypes separately (described in the subsequent sections below) and sums them up to derive the total number of infected donors in the US.

A-IV.B.2.a.v.(1) 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 from a donor. Source Plasma donors donate an average of 14 times per year, but can donate up to 48 times per year. This section of the model estimates the number of US Source Plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996. The three genotypes are calculated separately. The following equations summarized the calculation for MM genotype. Calculations for MV and VV genotypes are similar.

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}MM(age)y,i}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}MV(age)y,i}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}VV(age)y,i}$- Numbers of Source Plasma donors with either the MM. MV or VV genotype potentially infected with vCJD during travel to the UK during 1980-1996 by age, year and duration of travel.

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 (calculated in section A-IV. B. 2. a. ii.)

Variable: $Perc_{MM}$, $Perc_{MV}$, $Perc_{VV}$-the percentage of population who are either of the MM, MV or VV genotype (same as described in section A-IV.A.)

$$DR_{vCJD-S-UK-MM(age)y,i} = Bionomial(DR_{S-UK(age)y,i} \times Perc_{MM}, \Pr_{vCJD-MM-DR-UK(age)y,i}) \quad \text{(IV.B.2.a-15)}$$

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}MMy}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}MVy}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}VVy}$ - Number of Source Plasma donors who are either MM, MV or VV genotype and potentially infected with vCJD during travel/residency in the UK in year y.

$$DR_{vCJD-S-UK-MMy} = \sum_{Age=18-19\,yrs}^{50-54\,yrs} \sum_{i=1-30days}^{>=5\,years} DR_{vCJD-S-UK-MM(age)y,i} \quad \text{(IV.B.2.a-16)}$$

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.

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}MM\text{-}Defy}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}MV\text{-}Defy}$ $DR_{vCJD\text{-}S\text{-}UK\text{-}VV\text{-}Defy}$- Number of Source Plasma donors who are either of the MM, MV or VV genotype and potentially infected with vCJD in year y who met deferral criteria and calculated using the equation below:

$$DR_{vCJD-S-UK-MM-defy} = \sum_{Age=18-19\,yrs}^{50-45\,yrs} \sum_{i=3-5months}^{>=5\,years} DR_{vCJD-S-UK-MM(age)y,i} \quad \text{(IV.B.2.a-17)}$$

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}MM\text{-}Resy}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}MV\text{-}Resy}$, $DR_{vCJD\text{-}S\text{-}UK\text{-}VV\text{-}Resy}$- Residual risk due to the number of Source Plasma donors potentially infected with vCJD but not deferred by current policy

$$DR_{vCJD-S-UK-MM-Resy} = \sum_{Age=18-19}^{50-54\,yrs} \sum_{i=1-30days}^{>=3months} DR_{vCJD-S-UK-MM(age)y,i} \quad \text{(IV.B.2.a-18)}$$

Calculations for persons of the MV or VV genotypes are similar to those for MM genotype and are not repeated here.

A-IV.B.2.a.v.(2) Number of US Recovered Plasma donors with history of travel to the UK and potentially infected with vCJD during travel to the UK

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 section of the model estimates the number of US recovered plasma donors potentially infected with vCJD during travel to the UK from 1980 through 1996. The calculations for recovered plasma donors in this section are similar to the calculations for source plasma donors shown on section A-IV.B.2.a.v.(1).

A-IV.B.2.a.vi. Number of US 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. The 2006 version of risk assessment model assumed that vCJD infected individuals have infectious vCJD agent present in the blood during the last half of the incubation period. The updated 2010 FDA risk assessment assumed the vCJD infectious agent is likely present in the blood of an infected individual on average after 75% of the incubation period has elapsed and used a range of 50% - 90% to represent the uncertainty associated with the duration of the incubation period where infectious is present in the blood.

Variable: y - The calendar year in which a plasma donor traveled and was 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

$$T_{Inf-2002y} = 2002 - y \qquad \text{(IV.B.2.a-19)}$$

Variable: $I_{bl\text{-}MMy}, I_{bl\text{-}MVy}, I_{bl\text{-}VVy}$ –Index variable representing whether blood is infectious (and infectious agent is present in the blood of a donor who traveled in year y (yes=1, no=0).

Variable: $IP_{MM}, IP_{MV}, IP_{VV}$- Incubation periods for donors who are either MM, MV or VV genotypes (in years, same as described in section A-IV.A)

Variable: $IP_{inflbl\text{-}MM}, IP_{inflbl\text{-}MV}, IP_{inflbl\text{-}VV}$- Portions (percentages) of donors allocated by either MM, MV or VV genotypes and who are in the late stages of incubation period and infectious agent is present in blood.

If $T_{Inf\text{-}2002y} >= IP_{inflbl\text{-}MM} \times IP_{MM}$

Then, $I_{bl\text{-}MMy}=1$ (agent present in blood)

Otherwise, $I_{bl\text{-}MMy}=0$ (agent not present in blood) (IV.B.2.a-20)

A-IV.B.2.a.vi.(1). Number of US Source Plasma donors with a history of travel to the UK and potentially infected with vCJD and whose blood contains vCJD agent

This section 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: $DR_{vCJD\text{-}S\text{-}MM\text{-}UK\text{-}infbly}$, $DR_{vCJD\text{-}S\text{-}MV\text{-}UK\text{-}infbly}$, $DR_{vCJD\text{-}S\text{-}VV\text{-}UK\text{-}infbly}$-Annual number of source plasma donors of MM, MV ad VV genotypes with infectious agent in the blood

Variable: $DR_{vCJD\text{-}S\text{-}MM\text{-}UK\text{-}def\text{-}infbly}$, $DR_{vCJD\text{-}S\text{-}MV\text{-}UK\text{-}def\text{-}infbly}$$DR_{vCJD\text{-}S\text{-}VV\text{-}UK\text{-}def\text{-}infbly}$-Annual number of source plasma donors of MM, MV ad VV genotypes with infectious agent in the blood and are deferred based on current policy

Variable: $DR_{vCJD\text{-}S\text{-}MM\text{-}UK\text{-}res\text{-}infbly}$, $DR_{vCJD\text{-}S\text{-}MV\text{-}UK\text{-}res\text{-}infbly}$$DR_{vCJD\text{-}S\text{-}VV\text{-}UK\text{-}res\text{-}infbly}$-Annual number of source plasma donors of MM, MV ad VV genotypes with infectious agent in the blood and not deferred based on current policy

Equations for calculations of above variables for MM genotype were summarized below:

If $I_{bl\text{-}MMy}=1$,

Then,

$$DR_{vCJD-S-MM-UK-\text{inf}bly} = DR_{vCJD-S-UK-MMy} \quad \text{(IV.B.2.a-21)}$$

$$DR_{vCJD-S-MM-UK-def-\text{inf}bly} = DR_{vCJD-S-UK-MM-defy} \quad \text{(IV.B.2.a-22)}$$

$$DR_{vCJD-S-MM-UK-res-\text{inf}bly} = DR_{vCJD-S-UK-MM-resy} \quad \text{(IV.B.2.a-23)}$$

Otherwise,

$$DR_{vCJD-S-MM-UK-\text{inf}bly} = 0 \quad \text{(IV.B.2.a-24)}$$

$$DR_{vCJD-S-MM-UK-def-\text{inf}bly} = 0 \quad \text{(IV.B.2.a-25)}$$

$$DR_{vCJD-S-MM-UK-res-\text{inf}bly} = 0 \quad \text{(IV.B.2.a-26)}$$

The three variables below sum the above estimates over the year and genotypes, and were presented on the work sheet "Mode-IV. Exposure Assessment" for the subsequent calculation of the model.

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}infbl}$- Total number of source plasma donors whose blood contains vCJD agent

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}def\text{-}infbl}$- Total number of source plasma donors whose blood contains infectious agent and are deferred based on current policy

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}res\text{-}infbl}$ Total number of source plasma donors whose blood contains infectious agent and are not deferred based on current policy

A-IV. B.2.a.vi.(2). Number of US recovered plasma donors with history of travel to the UK and potentially infected and whose blood contains vCJD agent

This section 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. The calculations are similar to the calculation for source plasma donors described in section A-IV.B.2.a.vi.(1).

A-IV.B.2.b. US plasma donors with history of travel to France: Annual number potentially infected and whose blood may contain vCJD agent

This section estimated the numbers of donors who have a history of travel to France and estimates the numbers of donors who might have been infected during travel to France and whose blood contains vCJD agent in 2002. Detailed calculations are on the model worksheet "IV.B.2.b Travel-FR". The results of calculations are intermediate calculations contained in the model worksheet "Model-IV. Exposure Assessment" and are used subsequent calculations in the model.

Assumption used in the model: There was exposure risk for donors who traveled to France since 1980.

As mentioned in previous sections the FDA model assumes there is essentially no exposure risk for the donors who travel to the UK after 1996, since stringent human food chain controls were implemented in the UK in 1996. However, the FDA model assumed that there is exposure risk for the donors who travel to France after 1996, since there has no similar measures implemented in France.

A-IV.B. 2. b. i. US plasma donors with a history of travel to France: Percentage of donors and travel duration

In this section, blood donors are characterized by frequency and duration of travel to 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 less than 5 years . 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 for the years of 1997-2002 when necessary to estimate potential travel characteristics and risk beyond 1996. The calculations in this section are similar to the calculations for donors with history of travel to UK shown in section A-IV.B.2.a.i.

A-IV.B.2.b.ii. US plasma donors with a history of travel to France: Percentage and number of donors by age group, year of travel and duration of travel

This part of risk assessment calculates the annual number of US Source Plasma and recovered plasma donors that traveled to France by specific year(s) and for a specific duration of time since 1980 by age. The calculations in this section are similar to the calculations for donors with history of travel to UK shown in section A-IV.B.2.a.ii.

A-IV.B. 2.b.iii. US plasma donors with history of travel to France: Adjustment of the vCJD risk for France to account for variations in French 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 have a value of 1. The vCJD relative risk value of 1 represents a prevalence equivalent to 100% of the UK asymptomatic and symptomatic vCJD prevalence. Based on information in FDA guidance (2002), the relative vCJD risk value for France is 0.05 (French vCJD prevalence is 5% of the UK vCJD prevalence) because risk of exposure to BSE in France was smaller than the risk in the UK. The vCJD risk value is assigned based on factors such as domestic UK beef consumption, and the approximate 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 additionally approximately 5% of its beef was imported from the UK. As of August 2006, France reported 20 cases of vCJD in its human population. 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.B.2.b. iii. (1). US plasma donors with a history of travel to France: Average cumulative risk of individual resident of France since 1980

Variable: R_{FR} – The cumulative risk of individual residents of France from 1980 till present

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.B.2.b.iii.(2). 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.B.2.a iii.)

Variable: BSE_{UKy} (same variable used in A-IV.B.2.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.B.2.b.iii.(3). 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*

Calculations for this section are similar to the calculations for the donors who traveled to the UK shown in section A-IV.B.2.a.iii.(3).

A-IV.C. 1. b. iv. US plasma donors with history of travel to France: Probability of vCJD infection for donor based on year, duration of travel, age and genotype

This section describes the portion of the model that estimates the potential probability that a US plasma donor in a specific age group of a specific genotype who traveled to France in a specific year for a specific duration since 1980 was infected with vCJD. Calculations for this section are similar to the calculations for the donors who traveled to the UK shown in section A-IV.B.2.a.iv.

A-IV.B. 2.b.v. Total number of all US plasma donors with a history of travel to France: Number potentially infected with vCJD

Calculations for this section are similar to the calculations for the donors who traveled to the UK shown in section A-IV.B.2.a.v.

A-IV.B.2.b.vi. Number of US Plasma donors with history of travel to France and potentially infected and vCJD agent is present in the blood

Calculations for this section are similar to the calculations for the donors who traveled to the UK shown in section A-IV.B.2.a.vi.

A-IV.B.2.c. US plasma donors with history of travel to other countries in Europe: Annual number potentially infected and whose blood may contain vCJD agent

This section estimated numbers of donors who have a history of travel to other countries in Europe, other than the UK and France, and numbers of donors who might have been infected during travel in these regions and whose blood may contain vCJD agent in 2002. Detailed calculations are shown on the model worksheet "IV.B.2.c Travel-EU". The results of calculations are drawn from the model worksheet "Model-IV. Exposure Assessment" and used to perform subsequent calculations for the model.

Assumption used in the model: There was exposure risk for donors who traveled to the other countries in Europe since 1980.

With similar reason, FDA model considered there is exposure risk for donors who traveled to other countries in Europe after 1996 as those traveled to France.

A-IV.B. 2.c. i. US plasma donors with a history of travel to other countries in Europe: Percentage of US donors and travel duration

In this section, blood donors are characterized by frequency and duration of travel to other countries in Europe 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 Europe since 1980, and to derive the frequencies for various durations of travel for less than 5 years. 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 for the years of 1997-2002 when necessary to estimate potential travel characteristics and risk beyond 1996. The calculations in this section are similar to the calculations for donors with history of travel to the UK are shown in section A-IV.B.2.a.i.

A-IV.B.2.c.ii. US plasma donors who traveled to other countries in Europe: Total number by year of travel, duration of travel and by age group

This section of the risk assessment calculates the annual number of US plasma donors who traveled to other countries in Europe (other than the UK and France) since 1980. The calculations in this section are similar to the calculations for donors with history of travel to UK shown in section A-IV.B.2. a.ii.

A-IV.B.2.c.iii. US plasma donors who traveled to other countries in Europe: Adjustment of vCJD risk to account for variations in EU BSE risk by duration of travel and 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 have a value of 1, the vCJD risk value for France was assumed to be 0.05. Based on information in FDA guidance (2002) FDA model also assumed vCJD risk value for other countries in Europe was 0.015. The vCJD 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). Other 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. Current US vCJD geographic deferral policy defers blood

donors with a history of residence in other 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.

The calculations in this section are similar to the calculations for donors with history of travel to France shown in section A-IV.B.2. B. iii.

A-IV. B. 2. c. iv. US plasma donors with a history of travel to other countries in Europe: Probability of vCJD infection based on year, duration, age and genotype

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 other countries in Europe for a specific duration since 1980 was infected with vCJD. The calculations in this section are similar to the calculations for donors with history of travel to the UK shown in section A-IV.B. 2. a. iv. and are not shown.

A-IV.B.2.c.v. Number of US plasma donors with a history of travel to other countries in Europe: Number potentially infected with vCJD.

The calculations in this section are similar to the calculations for donors with history of travel to the UK shown in section A-IV.B.2.a.v.

A-IV. B.2.c.vi. Number of US plasma donors who traveled to other countries in Europe: Number potentially infected and whose blood contains vCJD agent

The calculations in this section are similar to the calculations for donors with history of travel to the UK shown in section A-IV.B. 2. a. vi.

A-IV.B.2.d. US plasma donors deployed by the military in the UK or other countries in Europe: Annual number potentially infected and whose blood contains vCJD agent

This section of the model estimated the number of donors who might have been infected with vCJD when they were military personnel or dependents deployed to US military bases in the UK, France and other countries in Europe during the period from 1980 through 1996. Detailed calculations are on the model worksheet "IV.B.2.d. Military". The results of the calculations refer to the model main worksheet "Model-IV. Exposure Assessment" and are used for subsequent calculations in the model.

All donors who have been deployed in the UK or other countries in Europe are deferred by current policy. Current donor deferral policy indefinitely defers donors who have been deployed to the military bases in the UK or other countries in Europe for more than six months.

A-IV.B.2.d.i. US plasma donors deployed by Military: Percentage of US plasma donors deployed at US military bases during the years 1980 through 1996

Variable: $Perc_{DR\text{-}DOD}$ - Percentage of US blood donors who were military residents in other countries in Europe for ≥ 6 months 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 other countries in Europe after 1996 was assumed to be negligible, because it is assumed that most of the risk for military personnel posted in Europe 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.

Assumption used in the model: Approximately 3% of US blood donors have been military residents in European countries between 1980 and 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.
- Source plasma donors would have similar donation demographics and characteristics as whole blood donors.

A-IV. B. 2. d. ii. US plasma donors deployed by Military: Number of donors by year of deployment since 1980

A-IV.B.2.d.ii.(1). US plasma donors deployed by Military: Number of Source Plasma 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.

Variable: $Perc_{DR\text{-}DODy}$ – Percentage of Source Plasma donors who have a history of military deployment in Europe in a specific year y.

$$Perc_{DR-DODy} = (DOD_y / \sum_{y=1980}^{1996} DOD_y) \times 100\% \qquad \text{(IV.B.2.d-1)}$$

Variable: age - age of donors were 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.B.2.a.ii.)

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. B. 2. d. i)

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

The estimated annual number of Source Plasma donors who have a history of military deployment in Europe by age is represented by the equation:

$$DR_{S-DOD(age)} = DR_{S(age)} \times Perc_{DR-DOD} \quad \text{(IV.B.2.d-2)}$$

Variable: $DR_{S\text{-}DOD(age)y}$ – Number estimated annual number of Source Plasma donors who resided on military bases in Europe by age and deployment year

$$DR_{S-DOD(age)y} = DR_{S-DOD(age)} \times Perc_{DR-DODy} \quad \text{(IV.B.2.d-3)}$$

A-IV. B. 2. d. ii. (2). US plasma donors deployed by Military: Number of recovered plasma donors deployed by year

The calculations in this section are similar to the calculations for source plasma donors shown in section A-IV.B. 2. d. ii. (1).

A-IV.B.2.d.iii. US plasma donors deployed by Military: 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

Assumption used in the model: The relative risk value of US military residents in other countries 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).

Assumption used in the model: It was assumed that there was a two consecutive years of residence on a base for each deployment.

Variable: y – Year of deployment (same variable used above in A-IV.B.2.a.iii)

Variable: y_{epi} – The specific year of BSE epidemic in the UK (same variable used above in A-IV.B.2.a. iii).

Variable: BSE_{UKy} - Number of diagnosed BSE cases in the UK by year from 1980 through 1996 (same variable used above in A-IV.B.2.a.iii)

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 other countries in Europe 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_{Base} - vCJD risk for donors that resided on US military bases in Europe throughout the period between 1980 and 1996

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.

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:

- It was assumed that the vCJD risk was additive and can be prorated on a yearly basis.
- The vCJD risk in a specific year was assumed to be proportional to the reported number of 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:

$$R_{Basey} = R_{Base} \times BSE_{UKy} / \sum_{y=1980}^{1996} BSE_{UKy} \qquad \text{(IV.B.2.d-4)}$$

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.

Assumption used in the model: The model assumed an average of two consecutive years of deployment:

$$R_{DR\text{-}DODy} = R_{Basey} + R_{Base(y+1)} \qquad \text{(IV.B.2.d-5)}$$

A-IV.B.2.d.iv. US plasma donors deployed by the Military: Estimation of probability of vCJD infection for an individual plasma donor by year of deployment, age and genotype

The calculations in this section are similar to the calculations for donors who traveled to the UK shown in section A-IV.B.2.a.iv.

A-IV. B. 2. d. v. US plasma donors deployed by Military: 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

This section estimates the number of Source plasma and recovered plasma donors with a history of deployment in the UK or other countries in Europe during the period from 1980 through 1996, and potentially infected with vCJD. The calculations in this section are similar to the calculations for donors who traveled to the UK shown in section A-IV.B.2.a.v.

A-IV.B.2.d.vi. US plasma donors deployed by the Military: Potential number of donors with whose blood contains vCJD agent

The calculations in this section are similar to the calculations for donors who traveled to the UK shown in section A-IV.B.2.a.vi. and are not shown.

A-IV.B.2.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 in the New York City metropolitan area and possibly in other areas on the east coast of the US. To our knowledge there are no specific data available for plasma donors, therefore, data for blood donors was used in this risk assessment. Detailed calculations are on the model worksheet "IV.B.2.e. Euroblood". The results of the calculations are derived from model main worksheet "Model-IV. Exposure Assessment" and are used for subsequent calculations in the model..

A-IV.C.1.e.i. Annual number of US plasma donors who have been Euroblood recipients

A-IV.C.1.e.i. (1) annual number of US plasma donors who had received Euroblood

Variable: DR_{Tot} - (calculated in section A-IV.B.1.) 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.1.) 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

$$DR_{Eurob} = DR_{Tot} \times Perc_{DR-Eurob} \qquad \text{(IV.B.2. e-1)}$$

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

$$DR_{S-Eurob} = DR_S \times Perc_{DR-Eurob} \qquad \text{(IV.B.2.e-2)}$$

Variable: $DR_{R\text{-}Eurob}$ - Annual number of recovered plasma donors who were Euroblood recipients – represented by the equation:

$$DR_{R-Eurob} = DR_R \times Perc_{DR-Eurob} \quad \text{(IV.B.2.e-3)}$$

A-IV.C.1.e.i.(2) Annual number of Euroblood transfused into US plasma donors and number of European donors who donated blood

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:

$$Perc_{DN-UK(age)} = DN_{Bl-UK(age)} / \sum_{age=18}^{\geq 65} DN_{Bl-UK(age)} \quad \text{(IV.B.2.e-4)}$$

Variable: $EUBL_{(age)}$ – Number of 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

$$EUBL_{(age)} = EUBL_{Tot} \times Perc_{DN.-UK(age)} \quad \text{(IV.B.2.e-5)}$$

Variable: $DR_{EUBL(age)}$ – The number of European donors were grouped by age in five-year increments (e.g., 20-24 yrs, and so on) and the 18-19 yr old group that 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:

$$DR_{EUBL(age)} = EUBL_{(age)} \quad \text{(IV.B.2.e-6)}$$

A-IV.B.2.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 model estimated 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. Since calculations were essentially similar for all three

genotypes, and for the sake of brevity, only calculations for MM genotype are shown below as an example:

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 and is same variable as used in A-IV.C.1.c.iii.

Variable: $Pr_{Asym\text{-}vCJD\text{-}UK\text{-}MM(age)}$ - Probability of infection for individual UK resident of a specific age group of MM genotype and is(same variable as used in A-IV.C.1.c.iv..

Variable: $Pr_{vCJD\text{-}EU\text{-}MM(age)}$, $Pr_{vCJD\text{-}EU\text{-}MV(age)}$, $Pr_{vCJD\text{-}EU\text{-}VV(age)}$-Probability of infection for an individual European resident of a specific age group of MM, MV or VV genotypes.

Assumption used in the model: Probability of infection is proportional to the risk of exposure:

$$\Pr_{vCJD-EU-MM(age)} = \Pr_{asym-vCJD-UK-MM(age)} \times R_{EU}$$

(IV.B.2.e-7)

Variable: $DR_{vCJD\text{-}EUBL\text{-}MM(age)}$, $DR_{vCJD\text{-}EUBL\text{-}MV(age)}$, $DR_{vCJD\text{-}EUBL\text{-}VV(age)}$-Annual number of infected European donors of MM, MV or VV genotypes 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.B. 2. e. i) 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. B. 2. e. ii) as parameters of the distribution.

$$DR_{vCJD-EUBL-MM(age)} = Bionomial(DR_{EULL(age)} \times Perc_{MM}, \Pr_{vCJD-EU-MM(age)})$$

(IV.B.2.e-8)

Variable: $EUBL_{vCJD}$ - Total units of Euroblood, collected from a vCJD infected donor, 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 Euroblood containing vCJD agent.

$$EUBL_{vCJD} = \sum_{age=18-19}^{>65} DR_{vCJD-EUBL-MM(age)} + \sum_{age=18-19}^{>65} DR_{vCJD-EUBL-MV(age)} + \sum_{age=18-19}^{>65} DR_{vCJD-EUBL-VV(age)}$$

(IV.B.2.e-9)

A-IV. B. 2. e. iii. Annual number of plasma donors potentially infected with vCJD via transfusion with Euroblood

A-IV.B.2.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

$$\Pr_{vCJD-EUBL} = EUBL_{VCJD} / EUBL_{Tot} \qquad \text{(IV.B.2.e-10)}$$

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 from a vCJD-infected donor.

$$\Pr_{vCJD-EUBL-recip} = 1 - Binomdist(0, EUBL_{avg}, \Pr_{vCJD-EUBL}, false) \qquad \text{(IV.B.2.e-12)}$$

The Excel function Binomdist (n, N, p, false) calculates the probability of n "successful" outcomes in a test, if the outcome of each trial of the test is either a "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.B.2.e-12, $Binomdist(0, EUBL_{avg}, Pr_{vCJD\text{-}EUBL}, false)$ 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 Plasma and recovered plasma donors who have received Euroblood ($DR_{S\text{-}EUBL}$ and $DR_{R\text{-}EUBL}$ estimated in section A-IV.B.2.e.i.) and the probability of infection for the individual Euroblood recipient ($Pr_{vCJD\text{-}EUBL\text{-}receip}$ estimated in section A-IV.B.2.e.ii) 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 infected due to transfusion with a Euroblood unit:

$$DR_{vCJD-S-EUBL} = Binomial(DR_{S-EUBL}, \Pr_{vCJD-EUBL-recip}) \qquad \text{(IV.C.1.e-13)}$$

Variable: $DR_{vCJD\text{-}R\text{-}EUBL}$ - Annual number of recovered plasma donors possibly infected via transfusion with a unit of Euroblood

$$DR_{vCJD-R-EUBL} = Binomial(DR_{R-EUBL}, \Pr_{vCJD-EUBL-recip}) \qquad \text{(IV.C.1.e-14)}$$

Variable: $DR_{vCJD\text{-}EUBL}$ - Annual number of all plasma donors possibly infected through transfusion with a unit of Euroblood

$$DR_{vCJD-EUBL} = DR_{vCJD-S-EUBL} + DR_{vCJD-R-EUBL} \quad \text{(IV.B.2.e-15)}$$

Assumption used in the model: No Euroblood Recipient is deferred under current policy

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:

$$DR_{vCJD-EUBL-Def} = 0 \quad \text{(IV.B.2.e-16)}$$

$$DR_{vCJD-EUBL-Res} = DR_{vCJD-EUBL} \quad \text{(IV.B.2.e-17)}$$

A-IV.B.2.e.iii.b. Annual number of plasma donors that received Euroblood and are potentially infected and whose blood contains the vCJD agent

Assumption used in the model: All infected Euroblood recipients have vCJD agent present in their blood

Variable: $DR_{EUBL\text{-}Infbl}$ - Annual number of plasma donors infected via transfusion using Euroblood and whose blood contained vCJD agent in 2002

$$DR_{EUBL-Infbl} = DR_{vCJD-EUBL} \quad \text{(IV.B.2.e-18)}$$

A-IV.B.2.f. Total number all plasma donors who may potentially be infected with vCJD and the vCJD agent may be present through all sources of exposure

This section sums the number of plasma donors who might have been exposed to vCJD through different sources. The calculations from this point forward are on the model worksheet "Model-IV. Exposure Assessment".

Variable: $DR_{Def\text{-}infbl}$ - Estimated annual number of Plasma donors potentially infected with vCJD and having vCJD agent present in blood and plasma that are deferred by current policy

Variable: $DR_{UK\text{-}Def\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD in the UK and whose blood contains vCJD agent that are deferred by current policy

Variable: $DR_{FR\text{-}Def\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD in France and whose blood contains vCJD agent that are deferred by current policy

Variable: $DR_{DOD\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD in the military base and whose blood contains vCJD agent that are deferred by current policy.

$$DR_{Def\text{-}infbl} = DR_{UK\text{-}Def\text{-}infbl} + DR_{FR\text{-}Def\text{-}infbl} + DR_{DOD\text{-}infbl} \qquad \text{(IV.B.2.f-1)}$$

Assumption about variable: This population includes the potential Source Plasma and recovered plasma donors and whose blood and plasma contains vCJD agent that have long-term travel history to the UK (≥3 mo), and France (≥ 5 yrs); and all the donors that have a history of military deployment (or military dependent, etc.) in Europe from 1980 – 1996.

Variable: $DR_{Res\text{-}infbl}$ - Estimated annual number of Plasma donors potentially infected with vCJD with agent present in blood and plasma and are not deferred by deferral policy.

Variable: $DR_{UK\text{-}Res\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD in the UK and having vCJD agent and whose blood contains vCJD agent and are deferred by current policy

Variable: $DR_{FR\text{-}Res\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD and having vCJD agent and whose blood contains vCJD agent and are deferred by current policy

Variable: $DR_{Eurobl\text{-}infbl}$ - Estimated annual number of Plasma donors potentially exposed to vCJD and having vCJD agent and whose blood contains vCJD agent and are deferred by current policy

$$DR_{Res\text{-}infbl} = DR_{UK\text{-}Res\text{-}infbl} + DR_{FR\text{-}Def\text{-}infbl} + DR_{DOD\text{-}infbl} \qquad \text{(IV.B.2.f-2)}$$

Assumption about variable: This population includes the potential Source Plasma and recovered plasma donors with vCJD agent present in blood and plasma that have short-term travel history to the UK (< 3 mo), France (< 5 yrs), and a history of receiving Euroblood.

A-IV.B.3. Annual number of all US plasma donors potentially infected with vCJD and whose blood may contain vCJD agent and who may not be deferred by questionnaire screening

This section calculated the number of plasma donors with vCJD agent in the blood and who 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. Uniform distribution (0.85, 0.99) was used to represent the range of uncertainty associated with Effectiveness of US donor deferral policy.

A-IV.B.3.a. US plasma donors with a history of travel to the UK

Variable: $DR_{vCJD\text{-}S\text{-}UK\text{-}inf bl\text{-}NR}$ - Annual number of potential Source Plasma donors with history of travel to the UK, with vCJD agent present in blood and plasma, and were not removed by deferral screening.

Variable: $DR_{vCJD\text{-}R\text{-}UK\text{-}inf bl\text{-}NR}$ - Annual number of potential recovered plasma donors with history of travel to the UK, with vCJD agent present in blood and plasma, and were not removed by deferral screening.

Assumption used in model: This includes potential Plasma donors with vCJD agent present in blood and plasma who did not meet deferral criteria or who meet deferral criteria but for a variety of reasons are not deferred.

$$DR_{vCJD-S-UK-\inf bl-NR} = DR_{vCJD-S-UK-Res-\inf bl} + DR_{vCJD-S-UK-Def-\inf bl} \times (1 - Eff_{Def}) \qquad \text{(A-IV.B-1)}$$

$$DR_{vCJD-R-UK-\inf bl-NR} = DR_{vCJD-R-UK-Res-\inf bl} + DR_{vCJD-S-UK-Def-\inf bl} \times (1 - Eff_{Def}) \qquad \text{(A-IV.B-2)}$$

A-IV.B.3.b. US plasma donors with a history of travel to France.

Calculations for this section are similar to the calculations for donors with a history of travel to the UK shown in section A-IV.B.3.a.

A-IV.B.3.c. US plasma donors with a history of travel to other countries in Europe.

Calculations for this section are similar to the calculations for donors with a history of travel to the UK shown in section A-IV.B.3.a.

A-IV. B. 3. d. US plasma donors with a history of deployment to military bases in Europe.

Calculations for this section are similar to the calculations for donors with a history of travel to the UK shown in section A-IV.B.3.a.

A-IV.B.3.e. U.S. plasma donors who have been Euroblood recipients.

Calculations for this section are similar to the calculations for donors with a history of travel to the UK shown in section A-IV.B.3.a.

A-IV.B.3.f. All U.S. plasma donors potentially with vCJD agent in the blood and who may not be deferred by questionnaire screening

This section sum up the vCJD donors of Source Plasma and recovered plasma exposed through different sources.

A-IV.B.4. Probability that a US plasma donor's blood potentially contained vCJD agent

Variable: $Pr(DR_{S\text{-}inf bl})$, $Pr(DR_{R\text{-}inf bl})$ - Probability a Source Plasma or recovered plasma donor contain vCJD agent in the blood

Variable: $DR_{vCJD\text{-}S\text{-}inf bl\text{-}NR}$, $DR_{vCJD\text{-}R\text{-}inf bl\text{-}NR}$- Source Plasma or recovered plasma donors who potentially contained vCJD agent in the blood, but not deferred by donor screening.

Variable: DR_S, DR_R -Annual number of Source plasma or recovered plasma donors (calculated in A-IV.B.1)

$$Pr(DR_{S-inf\,bl}) = DR_{vCJD-S-inf\,bl-NR} / DR_S \qquad \text{(A-IV.B.4.-1)}$$

Calculations for the probability a recovered plasma donor containing vCJD agent in the blood are similar to the calculation for source plasma donors shown above.

A-IV.C. Estimation of annual number and percentage of plasma pools potentially containing vCJD agent

A-IV.C.1. Probability that a plasma pool may contain vCJD donations

A-IV.C.1.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 Plasma 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: $DR_{pool\text{-}S}$, $DR_{pool\text{-}R}$ -Size of Source Plasma or recovered Plasma pool (donors/pool)

Data used in the model: Information for Source Plasma pool size was collected by the FDA from pdFVIII manufacturers. FDA is unable to show the complete data provided by manufacturers since the information is confidential, so only necessary, relevant summary information on various pools and their sizes is provided. The size of Source Plasma pools ranged from 6,000 donors per pool to 60,000 donors per pool with mean of -(b)(4)- 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. Again, FDA is unable to show the complete data provided by manufacturers since the information is confidential. 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: $n_{vCJD\text{-}DR\text{-}pool}$ - Designated number of vCJD donors in a single plasma pool.

Assumption used in the model: The number of vCJD donors 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 donors in a pool.

Variable: $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$, $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}R})$- Probability a Source or Recovered Plasma pool containing $n_{vCJD\text{-}DN\text{-}pool}$ ($n_{vCJD\text{-}DR\text{-}pool}$ =0, 1, 2, 3, 4) number of infected donors

$Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$ was determined by binomial density function, Binomdist($n_{vCJD\text{-}DR\text{-}pool}$, $DR_{pool\text{-}S}$, $Pr(DR_{S\text{-}infbl})$. Calculations of $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$ are similar to the calculation of $Pr(n_{vCJD\text{-}DR\text{-}pool\text{-}S})$.

A-IV.C.1.b. Probability a plasma pool may potentially contain a vCJD donor(s)

Variable: $Pr(vCJD\text{-}pool_S)$, $Pr(vCJD\text{-}pool_R)$-Probability of a Source or Recovered Plasma pool containing one or more vCJD donors

$$\Pr(vCJD\_pool_S) = 1 - \Pr(n_{vCJD-DR-poolS} = 0) \qquad \text{(IV.C.1-1)}$$

Calculation of probability for the recovered plasma pool is similar to the calculation for Source Plasma pool shown above.

Variable: $Pr(vCJD\text{-}pool)$-The probability that a plasma pool (including Source Plasma and recovered plasma pools) contained one or more vCJD donors- The distribution for pool size (or number of donations per pool) incorporated information on pool size.

Variable: $Perc_S$, $Perc_R$ – Percentage of Source Plasma or recovered plasma pools used to manufacture pdFVIII in the US

Assumption used in the model: Estimates suggest that approximately -(b)(4)- of pdFVIII products were made from Source Plasma, and -(b)(4)- were made from recovered plasma..

Based on the assumptions above that Source Plasma pools are used more frequently in the manufacture of pdFVIII and, on average contain fewer donors, 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 Plasma and recovered plasma pool) containing vCJD agent is a probability weight based on the percentages of the two types of plasma pools (-(b)(4)- for Source Plasma and -(b)(4)- for recovered plasma pools) used to make pdFVIII. A discrete distribution (X1, X2; p1, p2) represents two discrete values for the probabilities that a pool may contain a vCJD donor, 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. *Pr(vCJD-pool)* is sampled from $Pr(vCJD\text{-}pool_S$ and $Pr(vCJD\text{-}pool_R$ using the discrete distribution:

$$Pr(vCJD-pools) = Discrete(Pr(vCJD\text{-}pool\text{-}s), Pr(vCJD\text{-}pool\text{-}R); Perc_S, Perc_R)) \quad \text{(IV.C.1-2)}$$

Or

$$Pr(vCJD-pools) = Discrete(Pr(vCJD\text{-}pool\text{-}s), Pr(vCJD\text{-}pool\text{-}R); \text{----(b)(4)----})) \quad \text{(IV.C.1-3)}$$

A-IV.C.2. 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 -(b)(4)- million units of pdFVIII was made and distributed in the US.

Variable: $Perc_S$, $Perc_R$ – Represents the percentage of pdFVIII assumed in the model to be made from Source Plasma or recovered plasma (same as variable used in A-IV.C.1.b.)

Variable: $IU_{FVIII\text{-}S}$, $IU_{FVIII\text{-}R}$ –The total annual number of units of pdFVIII made from Source Plasma or recovered plasma. The total annual number of units of pdFVIII made from Source Plasma is represented by the equation:

$$IU_{FVIII-S} = IU_{FVIII} \times Perc_s \quad \text{(IV.C.2-1)}$$

The calculation for the total annual number of units of pdFVIII made from recovered plasma is similar.

A-IV.C.3. Annual total number of all plasma pools used to make pdFVIII

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 -(b)(4)- of the total pdFVIII supply distributed annually in the US is manufactured from Source Plasma and -(b)(4)- 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 Plasma 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.C.3.a. Amount plasma per pool

Variable: $DN_{V\text{-}S}$, $DN_{V\text{-}S}$ – Volume of single unit Source Plasma or recovered plasma (ml).

Variable: $DR_{pool\text{-}S}$, $DR_{pool\text{-}R}$ – Number donors per Source Plasma or recovered plasma pool (same variable as used in A-IV.C.5).

Variable: $Freq_{DN\text{-}S}$, $Freq_{DN\text{-}R}$ –Average frequency of donations from a single plasma donor who contributed Source Plasma or recovered plasma for pdFVIII manufacture.

Data used in the model: The data for average number of units in a Source Plasma pool donated by a single donor was provided by blood centers. The Pert distribution (----(b)(4)---) was used to represent the average number of units in a Source Plasma pool from single donors, which is most likely -(b)(4)- and ranges from --(b)(4)--.

Assumption used in the model: All the plasma units in a recovered plasma pool comes from different donors. This is conservative assumption.

Variable: $V_{pool\text{-}S}$, $V_{pool\text{-}R}$ — Volume of a Source Plasma or recovered plasma pool (ml).

$$V_{pool-S} = DR_{pool-S} \times Freq_{DN-S} \times DN_{V-S} \quad \text{(IV. C. 3-1)}$$

Calculations of $V_{pool\text{-}R}$ are similar to the calculation of $V_{pool\text{-}S}$ shown above.

A-IV.C.3.b. Annual number of plasma pools used to manufacture pdFVIII in the United States

Variable: $IU_{FVIII\text{-}S}$, $IU_{FVIII\text{-}S}$ -Annual units of pdFVIII made from Source Plasma or recovered plasma (calculated in A-IV.C.2)

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: $Pool_S$, $Pool_R$–Annual number Source or Recovered Plasma pool used to make pdFVIII

$$Pool_s = Round((IU_s / Y_{avg}) / (V_{pool-s} / 1000)) \qquad \text{(IV. C. 3-2)}$$

Calculations of $Pool_R$ are similar as calculations of $Pool_S$ shown above.

A-IV. C. 4. Annual number vCJD plasma pools used to manufacture pdFVIII in the United States and percentage of contaminated pools

This section of the model estimated the annual number of vCJD plasma pool that may appear in an individual year. The annual number of vCJD Source and Recovered Plasma donors are estimated in section A-IV.B.3. In this section each individual vCJD donor is allocated to individual plasma pools by plasma types. Total number of vCJD plasma pools, percentage plasma pool containing vCJD agent and percentage contribution of vCJD vials from a specific pool are determined.

Assumption used in the model: The 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. The chance of a plasma pool containing plasma from more than one infected donors is negligible. The model assumed if more than one infected donors presented, they would present in different plasma pool.

Variable: $Pool_{vCJD\text{-}S}$, $Pool_{vCJD\text{-}R}$ – Annual number of Source Plasma or recovered plasma pools that contain vCJD agent used to make pdFVIII

Variable: $Pool_{vCJD}$ – Annual total plasma pools that contain vCJD agent used to make pdFVIII

Variable: $Pool_S$, $Pool_R$ –Annual number of Source Plasma or recovered plasma pools

Variable: $Perc_{vCJD\text{-}S\text{-}pool}$, $Perc_{vCJD\text{-}R\text{-}pool}$ -Percentage of Source Plasma or recovered plasma pools used to make pdFVIII that contains vCJD donations

$$Perc_{vCJD-S-pool} = (Pool_{vCJD-S} / Pool_S) \times 100\% \qquad \text{(IV.C.4-1)}$$

A-IV.D. Estimation of the Quantity of vCJD agent in a plasma pool that contains a donation from a donor infected with vCJD

A-IV.D.1. Quantity of vCJD agent present in a donation of a donor 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.

Variable: $A_{ic\text{-}iv}$-Conversion factor for iv ID_{50} from i.c. ID_{50}.

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. C. 3 for Source Plasma and same as $DN_{V\text{-}R}$ used in A-IV. C. 3 for Recovered Plasma)

Variable: I_{DN}-Quantity of vCJD agent in one donation of infected plasma (i.v. ID_{50}/ml)

$$I_{DN} = I_{bl} \times DN_V \times I_{Pl-perc} \times A_{iv-ic} \qquad \text{(IV.D.1-1)}$$

A-IV.D.2. Quantity of vCJD agent in a plasma pool containing a donation from donor infected with vCJD

Variable: $DN_{vCJD\text{-}DR\text{-}pool}$- Number of donations from an infected plasma donor, which varies based on type of plasma donated. (same as $Freq_{DN\text{-}S}$ used in A-IV.C.3 for Source Plasma and same as $Freq_{DN\text{-}R}$ used in A-IV. C. 3 for recovered plasma)

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 that average number of donations from single donor ranges from -(b)(4)- donations per donor, with a most likely of -(b)(4)- average donations per donor. For recovered plasma, it was assumed that the most likely number of donations per donor was only 1.

Variable: I_{Pool}- Initial infectivity in an infected plasma pool is represented by the equation:

$$I_{Pool} = I_{DN} \times DN_{vCJD-DR-pool} \qquad \text{(IV.D.2-1)}$$

MODULE 3 (IV.E)– CLEARANCE OF vCJD INFECTIVITY DURING MANUFACTURE OF pdFVIII

A-IV. E. Clearance of vCJD infectivity during manufacture of pdFVIII

A-IV.E.1. Estimated quantity of vCJD agent per IU FVIII product made from a specific vCJD plasma pool

The FDA model employed two stratifications of clearance:

- 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 two levels of reduction that may be achieved: a lower 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: DR_{Pool}- Size of plasma pool (number of donors/pool). Same as $DR_{pool\text{-}S}$ used in A-IV.C.3 for Source Plasma and same as $DR_{pool\text{-}R}$ used in A-IV.C.3 for Recovered Plasma)

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 Plasma and recovered plasma pool based on the data provided by pdFVIII manufacturers.

Variable: I_{Pool}- Initial infectivity in a specific infected plasma pool (calculated in A-IV.D.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

$$I_{Pool-Ap} = I_{Pool} / 10^{R_{Log}} \qquad \text{(IV.E-1)}$$

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.1.)

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)

$$I_{IU} = (I_{Pool-Ap} / (DR_{Pool} \times DN_{DR-Avg} \times DN_V)) \times 1000 / Y_{FVIII} \qquad \text{(IV.E-2)}$$

A-IV.E.2. Estimated percentage of FVIII vials that contain vCJD agent

Variable: $Perc_{vCJD\text{-}S\text{-}vial}$, $Perc_{vCJD\text{-}R\text{-}vial}$- Percentage vials made from Source Plasma and recovered plasma containing vCJD infectious agent.

Variable: $Perc_{vCJD\text{-}vial}$ – Overall percentage of vials containing vCJD infectious agent.

Assumption used in the model: Percentage of vials of pdFVIII containing vCJD infectious agent is same as the percentage pools containing vCJD infectious agent calculated in IV-C.4 for corresponding types of plasma.

Module 4 (IV.F): FVIII utilization and annual exposure

A-IV.F. pdFVIII utilization by HA and vWD patients and potential exposure to the vCJD agent

A-IV.F.1. Estimate of annual number of vCJD vials used by individual patient

A-IV.F.1.a. Patients with severe Hemophilia A disease

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 CDC and the six state Hemophilia Surveillance System project conducted from 1993-1998 collected a total of 17,848 records, each record representing a single year of medical data for a single HA patient. 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 comprehensive study collected standardized information on patient demographics, clinical treatment and outcome data. 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. The distributions were truncated by minimum and maximum FVIII usage of each subpopulation. 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	90% CI
Prophylaxis	No Inhibitor	578	164394 IU	(13574, 518781)	1.5159	10.02	(300, 1200000)	157949	(21000, 382,000)
	With Inhibitor – No Immune Tolerance	63	198781	(7859, 937480	1.4640	6.2861	(2000, 1000000	190523	(27000, 448000)
	With Inhibitor – With Immune Tolerance	62	569707	(14315, 3222471)	0.8782	5.5081	(10000, 4000000)	558700	(33000, 1593000)
Episodic	No Inhibitor	946	90489	(3001, 345416)	0.9882	10.60	(0, 1000000)	85270	(4600, 245000)
	With Inhibitor	151	169710	(4099, 835729)	0.6950	3.6822	(2000, 1000000)	160458	(5000, 489000)

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.

$$Vial_{Tot} = IU_{Yr} / IU_{Vial} \qquad \text{(IV.F.1-1)}$$

Variable: $Perc_{vCJD\text{-}vial}$ – Percentage pdFVIII vials containing vCJD agent

Variable: $Vial_{vCJD}$ - Annual number of pdFVIII vials used by individual patient (vials/yr, person)

$$Vial_{vCJD} = Binomial(Vial_{Tot}, Perc_{vCJD\text{-}vial}) \qquad \text{(IV.F. 1-2)}$$

A-IV.F.1.b. FVIII Utilization in patients with severe von Willebrand disease

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**	**90% CI**
Young (≤15 yrs of age)									
Prophylaxis	9	16%	164193	(9200, 504625)	0.4523	0.9794	(9200, 504625)	165713	(9900, 454000)
Episodic	14	24%	11122	(1010, 41850)	0.3900	1.1973	(1010, 41850)	11045	(1020, 34350)
Adult (>15 yrs of age)									
Prophylaxis	17	29%	187538	(15000, 772800)	0.5741	1.9569	(15000, 7728000)	186880.	(17000, 540000)
Episodic	18	31%	845556	(1000, 293800)	0.5855	1.4097	(1000, 293800)	86923	(2200, 240000)

Calculations of annual number of vCJD vials taken by individual vWD patient are similar to the calculations for HA patients shown in section A-IV. F.1.a.

A-IV.F.2. Quantityof vCJD agent in pdFVIII vials

This section of the model randomly picked vCJD vials from different vCJD pools simulated in section IV.D.2. and determine the amount of vCJD infectious agent contained in each random vCJD vial,

Variable: I_{iu} - Quantity of infectivity in the pdFVIII product made from a specific infected pool (i.v. ID_{50} per IU) (calculated in section IV.F.)

A-IV.F.3. Estimation of annual exposure

This section of the model sums up the infectivity in all vCJD vials taken by individual patent during a one-year period.

A-IV.F.3.a. Patient with severe HA disease

Assumption used in the model: Infectivity varies for each individual vial taken by one patient during a one-year period. We did not considered the probability that patient buy a bulk package at a time, which may results in multiple vials from same vCJD pool, therefore with same infectivity

Variable: I_{yr} - Annual exposure to vCJD through use of pdFVIII (i.v. ID_{50}/yr, person)

$$I_{yr} = \sum_{i=1}^{Vial_{vCJD}} I_{iu} \times IU_{Vial} \qquad \text{(IV.F. 3-1)}$$

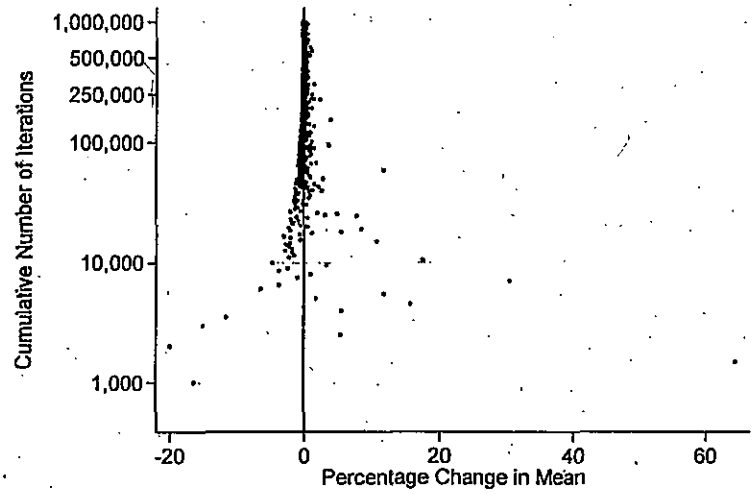
A-IV. F. 3. b. Patient with severe vWD

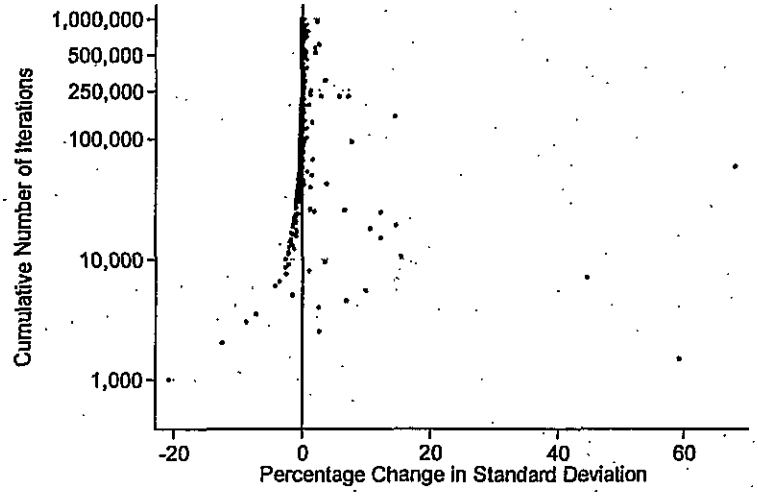
Calculations of annual exposure of individual vWD patient (i.v. ID50/yr, person) are similar to the calculations for HA patients shown in section A-IV. F.3.a.

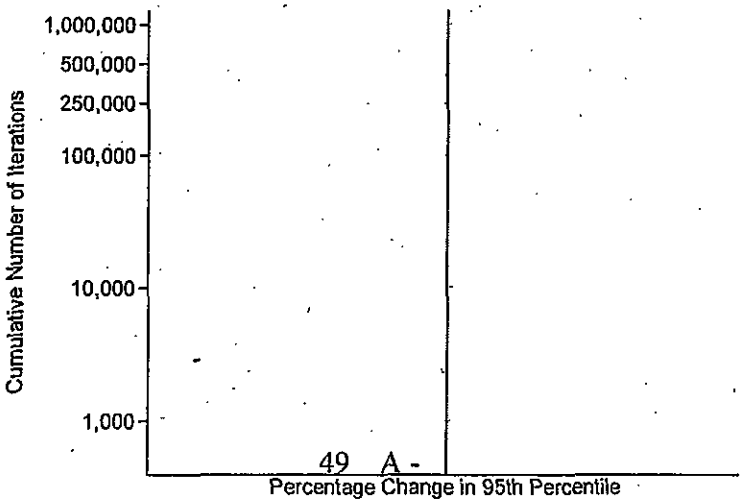
Testing Convergence of the Model

We ran a million iterations of the model. Convergence was analyzed by examining the percent change in the estimate of mean exposure for each 500 iteration block of the model, cumulatively. That is, the mean exposure was calculated for the first 500 iterations, 1,000 iterations, 1,500 iterations, etc., to 1,000,000 iterations. For the last 10,000 iterations, the average percentage change for the mean exposure of hemophilia patients with no inhibitor using the log 4-7 reduction assumption and the clinical prevalence assumption was 0.012 percent. The results are shown in Figure 1.

Figure 1. Convergence for risk estimates for hemophilia patient group with no inhibitor under assumption of 4-7 log reduction using low prevalence estimate for UK vCJD prevalence. The 95th percentile was always 0 under the low prevalence assumption.







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3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(2010/12/1～2011/2/28)

血対ID	ID	受理日	番号	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	適正措置報告
100269	2	2010/12/13	100749	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100270	3	2010/12/13	100750	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	有	無
100277	10	2010/12/15	100775	化学及血清療法研究所	乾燥スルホ化人免役グロブリン	スルホ化人免疫グロブリンG	ヒト血液	米国、日本	有効成分	有	有	無
100296	29	2011/2/22	100917	CSLベーリング	人血清アルブミン 破傷風抗毒素 フィブリノゲン 加第XIII因子	ヘパリン	ブタ腸粘膜	中国	製造工程	無	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（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第15回	15-1	感染症および寄生虫症	Ｂ型肝炎	米国	男性	58歳	2010/07/20	未回復	症例報告	外国製品	識別番号：10000017（完了報告、追加報告） 報告日：2010年9月6日、2010年9月22日 MedDRA：Version（13.0）
	15-2	肝胆道系障害	肝炎	米国	男性	14歳	不明	不明	症例報告	外国製品	識別番号：10000016（完了報告） 報告日：2010年8月13日 MedDRA：Version（13.0）
	15-2	臨床検査	抗ＨＢｃＩｇＧ抗体陽性	米国	男性	14歳	2010/05/29	不明	症例報告	外国製品	識別番号：10000016（完了報告） 報告日：2010年8月13日 MedDRA：Version（13.0）
	15-3	臨床検査	抗ＨＢｃＩｇＧ抗体陽性	スウェーデン	男性	不明	2009/11/16	回復	症例報告	外国製品	識別番号：10000011（完了報告） 報告日：2010年7月9日 MedDRA：Version（13.0）
	15-3	臨床検査	抗ＨＢｓ抗体陽性	スウェーデン	男性	不明	2009/11/16	回復	症例報告	外国製品	識別番号：10000011（完了報告） 報告日：2010年7月9日 MedDRA：Version（13.0）
	15-4	肝胆道系障害	肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号：10000007（完了報告） 報告日：2010年6月8日 MedDRA：Version（13.0）
	15-4	臨床検査	Ａ型肝炎抗体陽性	米国	女性	不明	2006	不明	症例報告	外国製品	識別番号：10000007（完了報告） 報告日：2010年6月8日 MedDRA：Version（13.0）
	15-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	不明	2006	不明	症例報告	外国製品	識別番号：10000007（完了報告） 報告日：2010年6月8日 MedDRA：Version（13.0）
	14-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号：10000002（追加報告） 報告日：2010年6月7日 第14回症例番号14-3において報告したものの追加報告 MedDRA：Version（13.0）

別紙様式第４

	番号	感染症の種類		発現國	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14回	14-1	臨床検査	抗ＨＢｃＩｇＧ抗体陽性	米国	男性	3歳	2010/01/28	不明	症例報告	外国製品	識別番号： 09000025（完了報告） 報告日：2010年2月26日 MedDRA: Version(12.1)
	14-2	肝胆道系障害	急性肝炎	米国	女性	75歳	2010/03/01	軽快	症例報告	外国製品	識別番号： 09000029（完了報告） 報告日：2010年3月24日 MedDRA: Version(13.0)
	14-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号： 10000002（完了報告） 報告日：2010年4月27日 MedDRA: Version(13.0)
	14-4	臨床検査	Ａ型肝炎陽性	カナダ	男性	19歳	不明	不明	症例報告	外国製品	識別番号： 10000008（完了報告） 報告日：2010年6月14日 MedDRA: Version(13.0)
第13回	13-1	臨床検査	Ｃ型肝炎陽性	米国	男性	65歳	2009/09	未回復	症例報告	外国製品	識別番号： 09000017（完了報告） 報告日：2009年11月5日 MedDRA: Version(12.1)
	13-2	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	32歳	2009/07/12	未回復	症例報告	外国製品	識別番号： 09000013（完了報告） 報告日：2009年9月24日 MedDRA: Version(12.1)
	13-3	感染症および寄生虫症	Ｂ型肝炎	米国	女性	40歳	2009/05	回復	症例報告	外国製品	識別番号： 09000012（完了報告） 報告日：2009年8月19日 MedDRA: Version(12.1)
	13-4	臨床検査	Ｂ型肝炎抗体陽性	米国	女性	37歳	2009/04/23	未回復	症例報告	外国製品	識別番号： 09000014（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)
	13-5	臨床検査	Ｂ型肝炎抗体陽性	米国	不明	新生児	2009/04/23	未回復	症例報告	外国製品	識別番号： 09000015（完了報告） 報告日：2009年10月8日 MedDRA: Version(12.1)

	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12回	12-1	感染症および寄生虫症	肝炎ウイルスキャリアー	米国	不明	不明	1993	不明	症例報告	当該製品	識別番号： 08000002（完了報告） 報告日：2008年12月22日 MedDRA: Version(11.1)
	12-2	感染症および寄生虫症	C型肝炎	米国	女性	48	2008/12/09	未回復	症例報告	外国製品	識別番号： 08000034（完了報告） 報告日：2009年 1月19日 MedDRA: Version(11.1)
	12-3	感染症および寄生虫症	C型肝炎	米国	女性	不明	不明	不明	症例報告	外国製品	識別番号： 09000004（完了報告） 報告日：2009年 5月18日 MedDRA: Version(12.0)
第11回	11-1	臨床検査	B型肝炎抗体陽性	米国	男性	17	2008/05	不明	症例報告	当該製品	識別番号： 08000007（完了報告） 報告日：2008年6月5日 MedDRA: Version(11.0)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号： 08000018（追加報告） 報告日：2008年11月12日 第11回症例番号11-2において10月17日に報告したものの追加報告 MedDRA: Version(11.1)
	11-2	感染症および寄生虫症	C型肝炎	米国	女性	不明	2008	不明	症例報告	当該製品	識別番号： 08000018（完了報告） 報告日：2008年10月17日 MedDRA: Version(11.0)
	11-3	感染症および寄生虫症	B型肝炎	スペイン	女性	不明	2008/6/3	未回復	症例報告	外国製品	識別番号： 08000026（完了報告） 報告日：2008年10月31日 MedDRA: Version(11.1)

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10回		0*	0	0	0	0	0	0	0	0	* 当該調査期間に対象となる感染症報告はなかった
第9回		0	0	0	0	0	0	0	0	0	
第8回		0	0	0	0	0	0	0	0	0	
第7回	7-1	臨床検査	ＨＩＶ抗体陽性	米国	不明	小児	不明	不明	症例報告	外国製品	識別番号： 06000022 (完了報告) 報告日：2006年8月24日 MedDRA: Version(9.0)
第6回	5-1	感染症および寄生虫症	C型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(追加報告) 報告日：2006年2月15日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの追加報告 MedDRA: Version(8.1)

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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5回	5-1	感染症および寄生虫症	C型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(追加報告) 報告日：2005年11月11日 MedDRA: Version(8.1)
	5-1	感染症および寄生虫症	C型肝炎	米国	男性	51歳	2005年9月	未回復	症例報告	当該製品	識別番号： 05000456(完了報告) 報告日：2005年10月27日 MedDRA: Version(8.1)
	1-3	感染症および寄生虫症	C型肝炎	米国	男性	26歳	2002/11/19	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	1-3	感染症および寄生虫症	B型肝炎	米国	男性	26歳	2002/10/4	不明	症例報告	当該製品	識別番号：03000006(追加報告) 報告日：2005年7月4日 第2回症例番号1-3において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年6月27日 第4回症例番号4-1において報告したものの追加報告 MedDRA: Version(8.0)

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４回	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年4月25日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-1 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(完了報告) 報告日：2005年4月7日 MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(追加報告) 報告日：2005年4月25日) MedDRA: Version(8.0)
	4-1	臨床検査	HTLV-2 血清学的検査陽性	フランス	男性	6歳	2005年	不明	症例報告	当該製品	識別番号： 05000001(完了報告) 報告日：2005年4月7日 MedDRA: Version(8.0)
	4-2	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	症例報告	外国製品	識別番号： 04000129 報告日：2005年3月31日) MedDRA: Version(8.0)

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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3回	3-1	感染症および寄生虫症	C型肝炎	米国	女性	37歳	2004/5/21	不明	症例報告	当該製品	識別番号：04000023 報告日：2004年6月30日 MedDRA: Version(7.0)
	3-2	臨床検査	B型肝炎抗体陽性	米国	女性	63歳	2004/7/27	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-2	臨床検査	A型肝炎抗体陽性	米国	女性	63歳	2004/8/16	不明	症例報告	当該製品	識別番号：04000059 報告日：2004年9月7日 MedDRA: Version(7.0)
	3-3	臨床検査	B型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)
	3-3	臨床検査	A型肝炎抗体陽性	米国	女性	50歳代	2004/9月	不明	症例報告	当該製品	識別番号：04000082 報告日：2004年10月20日 MedDRA: Version(7.1)

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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	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男性	26 歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2004 年 1 月 7 日 第 1 回症例番号 1-3 において報告したもの（FAX 報告）の完了報告 MedDRA: Version(6.1)
	2-2	感染症および寄生虫症	Ｃ型肝炎	ドイツ	女性	6 歳	1994/6/21	未回復	症例報告	外国製品	識別番号：04000013 報告日：2004 年 5 月 27 日 MedDRA: Version(7.0)
第１回	1-1	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：Ｄ03-31 報告日：2003 年 8 月 6 日 MedDRA: Version(6.1)
	1-2	臨床検査	Ｃ型肝炎ウイルス	米国	男性	不明	不明	未回復	症例報告	外国製品	識別番号：Ａ03-32 報告日：2003 年 8 月 6 日 MedDRA: Version(6.1)
	1-3	感染症および寄生虫症	Ｃ型肝炎	米国	男	26 歳	2003/8/30	軽快	症例報告	当該製品	識別番号：03000006 報告日：2003 年 11 月 28 日 FAX 報告：2003 年 11 月 19 日） MedDRA: Version(6.1)

100269	2	2010/12/13	100749	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100270	3	2010/12/13	100750	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	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 15 回	15-2	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	3ヶ月	2010/07	未回復	自発報告	当該製品	10000039、2 回(取下) 平成 22 年 9 月 13 日 MedDRA ver.13.0
	15-1	感染症および寄生虫症	A 型肝炎	ドイツ	女	66	2009/06	不明	自発報告	外国製品	10000012、1 回(完了;同一症例をアンチトロンビン III 番号 15-1 で報告) 平成 22 年 7 月 29 日 MedDRA ver.13.0
	15-1	感染症および寄生虫症	医薬品を介する感染因子の伝播	ドイツ	女	66	2009/06	不明	自発報告	外国製品	10000012、1 回(完了;同一症例をアンチトロンビン III 番号 15-1 で報告) 平成 22 年 7 月 29 日 MedDRA ver.13.0
	15-1	臨床検査	肝酵素上昇	ドイツ	女	66	2009/06	不明	自発報告	外国製品	10000012、1 回(完了) 平成 22 年 7 月 29 日 MedDRA ver.13.0
	15-1	臨床検査	A 型肝炎抗体陽性	ドイツ	女	66	2009/06	不明	自発報告	外国製品	10000012、1 回(完了) 平成 22 年 7 月 29 日 MedDRA ver.13.0
第 11 回	11-1	感染症および寄生虫症	C 型肝炎	日本	女	33	2008/7/20	未回復	自発報告	当該製品	08000486、3 回(完了;因果関係が否定されたため、報告対象外として完了報告) 平成 20 年 11 月 5 日 MedDRA ver.11.1
第 5 回	7	感染症および寄生虫症	C 型肝炎	日本	女	70	2005/6/10	回復	自発報告	当該製品	05000058、2 回(取下) 平成 17 年 7 月 19 日 MedDRA ver.8.0
	6	感染症および寄生虫症	サイトメガロウイルス性腸炎	日本	男	71	2005/5/21	軽快	自発報告	当該製品	05000049、2 回(取下) 平成 17 年 10 月 3 日 MedDRA ver.8.
第 4 回	5	感染症および寄生虫症	C 型肝炎	日本	女	28	2004/12	不明	自発報告	当該製品	04000290、3 回(取下) 平成 18 年 3 月 14 日 MedDRA ver.7.1

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	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4回	4	感染症および寄生虫症	ブドウ球菌感染	日本	女	1	2004/11/24	軽快	自発報告	当該製品	05000029、2回(完了) 平成17年7月19日 MedDRA ver.8.0
第3回	3	感染症および寄生虫症	C型肝炎	日本	男	79	2003/10	未回復	症例報告	当該製品	04000082、2回目(完了) 平成16年7月14日 MedDRA ver.7.0
第2回	2	感染症および寄生虫症	B型肝炎	日本	男	76	2003/8/4	未回復	症例報告	当該製品	03000113、1回目(完了) 平成16年2月4日 MedDRA ver.6.1
	1	感染症および寄生虫症	B型肝炎	日本	女	55	2004/1/9	未回復	症例報告	当該製品	03000111、2回目(完了) 平成16年4月9日 完了報告 MedDRA ver.6.1

100277	10	2010/12/15	100775	化学及血清療法研究所	乾燥スルホ化人免疫グロブリン	スルホ化人免疫グロブリンG	ヒト血液	米国、日本	有効成分	有	有	無

別紙(3)

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15回	1	感染症および寄生虫症	A型肝炎	ドイツ	女	66	不明	不明	症例報告	外国製品	識別番号3-10000013 報告日:2010年08月06日
	1	臨床検査	A型肝炎抗体陽性	ドイツ	女	66	2009/6	不明	症例報告	外国製品	識別番号3-10000013 報告日:2010年08月06日
	2	感染症および寄生虫症	HIV感染	ドイツ	男	31	2009/4	不明	症例報告	外国製品	識別番号3-10000026 報告日:2010年10月27日
第14回	1	感染症および寄生虫症	A型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
	1	感染症および寄生虫症	B型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
	1	感染症および寄生虫症	C型肝炎	ドイツ	女	71	2009/12/14	不明	症例報告	外国製品	識別番号3-09000024 報告日:2010年8月20日*
	2	感染症および寄生虫症	HIV感染	イタリア	男	28	2004/11	不明	症例報告	外国製品	識別番号3-09000026 報告日:2010年2月26日 報告対象外報告:2010年3月29日
第13回	報告なし										
第12回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	66	2009/5/1	不明	症例報告	外国製品	識別番号3-09000009 報告日:2009年07月22日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	77	2009/1/5	不明	症例報告	外国製品	識別番号3-08000039 報告日:2009年02月17日
	3	感染症および寄生虫症	C型肝炎	ドイツ	女	77	2009/1/5	不明	症例報告	外国製品	識別番号3-08000040 報告日:2009年02月17日
第11回	1	感染症および寄生虫症	HIV感染	ドイツ	男	35	不明	不明	症例報告	外国製品	識別番号3-08000029 報告日:2009年02月17日
	1	感染症および寄生虫症	B型肝炎	ドイツ	男	35	不明	不明	症例報告	外国製品	識別番号3-08000029 報告日:2009年02月17日
第10回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000026 報告日:2008年4月1日
	2	感染症および寄生虫症	B型肝炎	ドイツ	男	24	2008/1/10	不明	症例報告	外国製品	識別番号3-07000031 報告日:2008年3月25日
	3	臨床検査	C型肝炎抗体陽性	日本	女	37	2007/9/11	不明	症例報告	当該製品	識別番号1-07000251 報告日:2008年4月30日
	4	感染症および寄生虫症	C型肝炎	ドイツ	女	60	2007/4/13	不明	症例報告	外国製品	識別番号3-08000005 報告日:2008年5月29日
第9回	1	感染症および寄生虫症	B型肝炎	日本	女	33	2007/8/7	回復	症例報告	当該製品	識別番号1-07000093 報告日:2007年10月11日
第8回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007/1	不明	症例報告	外国製品	識別番号3-06000032 報告日:2007年3月30日
	1	臨床検査	C型肝炎陽性	ドイツ	女	61	2007/1	不明	症例報告	外国製品	識別番号3-06000032 報告日:2007年3月30日
第7回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日
	1	臨床検査	C型肝炎抗体陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告日:2006年12月20日

	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6回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	63	2005/11	不明	症例報告	外国製品	識別番号3-06000004 報告日:2006年5月18日
第5回	1	感染症および寄生虫症	B型肝炎	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号3-05000494 報告日:2005年12月27日
	1	感染症および寄生虫症	輸血後肝炎	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号3-05000494 報告日:2005年12月27日
	1	臨床検査	抗HBs抗体陽性	ドイツ	男	74	2005/10/21	死亡	症例報告	外国製品	識別番号3-05000494 報告日:2005年12月27日
	2	感染症および寄生虫症	B型肝炎	ドイツ	女	77	2005/9/28	未回復	症例報告	外国製品	識別番号3-05000493 報告日:2005年12月27日
第4回	1	感染症および寄生虫症	C型肝炎	ドイツ	不明	不明	不明	不明	症例報告	外国製品	識別番号3-04000125 報告日:2005年5月27日
	2	感染症および寄生虫症	ウイルス性肝炎	ドイツ	女	55	1995年	不明	症例報告	外国製品	識別番号3-04000122 報告日:2005年6月8日
第3回	1	臨床検査	C型肝炎陽性	ドイツ	男	68	2004/08	不明	症例報告	外国製品	識別番号3-04000088 報告日:2004年11月22日
第2回	報告なし										
第1回	1	感染症および寄生虫症	C型肝炎	フランス	男	57	2003/6/16	不明	症例報告	外国製品	識別番号D03-38 報告日:2003年9月4日 取り下げ報告:2003年11月7日
	2	感染症および寄生虫症	C型肝炎	ドイツ	男	不明	不明	後遺症	症例報告	外国製品	識別番号D03-40 報告日:2003年9月11日
	3	臨床検査	C型肝炎RNA陽性	ドイツ	女	71	2003/6/27	後遺症	症例報告	外国製品	識別番号D03-41 報告日:2003年9月11日
	4	感染症および寄生虫症	HIV感染	ドイツ	男	67	2000/4頃	後遺症	症例報告	外国製品	識別番号D03-47 報告日:2003年10月3日
	5	感染症および寄生虫症	C型肝炎	ドイツ	男	64	2003/7/2	後遺症	症例報告	外国製品	識別番号D03-51 報告日:2003年10月10日
	5	臨床検査	C型肝炎抗体陽性	ドイツ	男	64	2003/7/2	後遺症	症例報告	外国製品	識別番号D03-51 報告日:2003年10月10日
	5	臨床検査	C型肝炎RNA陽性	ドイツ	男	64	2003/7/2	後遺症	症例報告	外国製品	識別番号D03-51 報告日:2003年10月10日
	6	感染症および寄生虫症	サイトメガロウイルス感染	ドイツ	男	0	2003/6末	死亡	症例報告	外国製品	識別番号3-03000005 報告日:2003月11年19日
	6	臨床検査	サイトメガロウイルス抗体陽性	ドイツ	男	0	2003/6末	死亡	症例報告	外国製品	識別番号3-03000005 報告日:2003年11月19日
	6	臨床検査	サイトメガロウイルス抗体陽性	ドイツ	男	0	2003/6末	死亡	症例報告	外国製品	識別番号3-03000005 報告日:2003年11月19日
	6	臨床検査	サイトメガロウイルス検査陽性	ドイツ	男	0	2003/6末	死亡	症例報告	外国製品	識別番号3-03000005 報告日:2003年11月19日

＊:今回調査期間に追加報告を行った。

MedDRA /J Ver.13.1

100296	29	2011/2/22	100917	CSLベーリング	人血清アルブミン 破傷風抗毒素 フィブリノゲン 加第X III因子	ヘパリン	ブタ腸粘膜	中国	製造工程	無	有	無