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配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配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。

感染症定期報告概要

（平成２０年１月２３日）

平成１９年９月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

A　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

１　平成１９年９月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

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

感染症定期報告の報告状況(2007/9/1～2007/11/30)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70112	2007/09/28	70553	A型肝炎	J Med Virol 2007; 79: 356-365	1997-2005年に、デンマーク、ドイツ、オランダ、ノルウェー、スペイン、スウェーデンおよび英国で、男性同性愛者にA型肝炎が大流行した。このA型肝炎アウトブレイクに関連する株の遺伝子学的関連性を調べたところ、これらの国の男性同性愛者から得られた株の大部分はMSM1と名づけられた遺伝子型IAに属する近縁のクラスターを形成していた。同期間に他のリスク群では異なったHAV株が流行していたことから、特異的な株がヨーロッパの男性同性愛者間では流行していたことを示す。	
70112	2007/09/28	70553	B型肝炎	Transfusion 2007; 47: 1162-1171	日本赤十字のスクリーニングシステムでHBsAg及び抗B型肝炎コア抗原抗体が陰性であったHBV DNA陽性供血者26名において急性HBV感染におけるウイルスマーカーの動態を調べた。検出可能期間の中央値は、HBV DNAが個別NATで74日、MP NATで50日、HBsAgが42日であった。26名中6名は変異型ウイルスに感染し、うち3名ではHBsAgが検出できなかった。HBV NATは、MPで行ったとしても、HBsAg検査よりも効果的で、HBsAgウインドウ期前後の感染供血者を排除することができる。	1
70112	2007/09/28	70553	B型肝炎	Transfusion 2007; 47: 1197-1205	日本赤十字血液センターに保管されている1997-2004年の反復供血者の全供血の遡及調査を行い、ID-NATのみHBV陽性である血液由来の血液製剤の輸血によるHBV伝播リスクを検討した。HBV ID-NATを実施したHBV転換供血者の保管血液15,721本中158検体 (1.01%) が陽性であった。スクリーニングをすり抜けたHBc 抗体価の低いオカルトHBVキャリア由来の血液製剤を原因とするHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期の供血による伝播リスクよりも10倍以上低い。	2
70165	2007/11/29	70768	B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日	平成19年3月、輸血によるHBV感染が疑われるとの報告が千葉県赤十字血液センターにあった。因果関係の確認のために実施した当該輸血用血液製剤に係る保管検体個別NATは陰性であり、献血者追跡調査を行った。1名の献血者が平成19年1月にB型肝炎を発症したとの情報が得られ、調べたところ、献血者のHBV-DNAは患者のそれと塩基配列が一致した。20プールNAT陰性、HBV保管検体個別NAT陰性であったが、献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された症例であった。	3
70165	2007/11/29	70768	B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日 一般演題51	2004年8月よりNATスクリーニングのプールサイズを50から20に縮小した。大阪府赤十字血液センターで検出されたHBV-NAT陽性事例81人を基にプールサイズ縮小の効果等について解析を行った。プールサイズ縮小後に100コピー未満/mLのHBV-NAT陽性者の比率が高くなっていることから、縮小による効果があると思われた。追跡調査、遡及調査及び医師の面談等による総合的な解析によりHBV低濃度キャリアが疑われる献血者がプールサイズ縮小後に多く検出されていることが推察された。	4

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70125	2007/10/24	70644	C型肝炎	Transfusion 2007; 47: 1534-1539	2002年8月28日から2005年2月28日の間にカナダBritish Columbiaのカナダ血液サービス(CBS)に報告された輸血伝播性C型肝炎(TT-HCV)疑い症例について、2002年8月以降実施された公衆衛生局(PH)への届出による影響、ならびにCBSによるHCV遡及(LB)及び追跡(TB)調査の有効性を検討した。LB及びTB調査により多数のHCV感染患者が同定されたが、PHへの届出はほとんど効果がなく、LBまたはTB調査開始の遅延を招いた。	5
70112	2007/09/28	70553	E型肝炎	J Med Virol 2007; 79: 734-742	日本におけるアラニンアミノトランスフェラーゼ(ALT)高値供血者の無症候性E型肝炎感染の現況を調べた。日本赤十字血液センターでALT高値(61-476 IU/L)の献血者6700名の血清検体を検査したところ、479名(7.1%)の供血者が抗HEV IgG陽性であった。ALT≧201 IU/L群はHEV RNA有病率が有意に高かった。ウイルス血症を発症した供血者9名から得られたHEV分離ウイルスは遺伝子型3に分類された。ALT≧201 IU/Lの日本人の約3%はHEV株の無症候性感染を有することが示された。	
70148	2007/10/26	70684	E型肝炎	Neurology 2007; 69: 156-165	同種造血幹細胞移植(HSCT)後に急性大脳辺縁系脳炎を発症した患者9名の臨床、EEG、MRI、ならびに臨床検査特性を調べた。患者は、順行性健忘、不適切な抗利尿ホルモン分泌症候群、軽度CSF多球症、一時的なEEG異常(顕性、不顕性けいれん発作を反映する場合が多い)を特徴とした。MRIでは、T2、FLAIR、DWI画像にて、鉤、扁桃体、内側嗅領、海馬領域内に高信号域を認めた。PCRを用いた初回腰椎穿刺CSFの検査では9名中6名がHHV6陽性であった。CSF中のHHV-6と関連付けられる可能性がある。	6 (=16)
70112	2007/09/28	70553	E型肝炎	Vox Sanguinis 2007; 93(Suppl.1): P203	2005年1月-2006年4月に北海道で献血者のHEV-RNAスクリーニングを行った。388,119名のうち、男性33名(1/7,120)、女性22名(1/6,962)がHEV-RNA陽性で、genotype 3が優勢であった。55名中40名は献血時のHEV抗体陰性であり、後に陽性となった。HEV陽性者にはALT値が上昇した人もいたが自覚症状はなかった。HEV-RNAは献血後、最長37日間検出された。HEV陽性献血者由来の輸血を受けた患者7名のうち、少なくとも2名が感染した。	7
70112	2007/09/28	70553	E型肝炎	肝臓 2007; 48(Suppl.1): O-178	発症前からのウイルス血症の推移、肝炎発症から沈静化までの経過を観察しえた輸血後E型肝炎2例の症例報告である。1例は輸血21日目にHEV RNA (genotype 4)が検出され、44日目にピーク値を、もう1例は輸血後3日目にHEV RNA (genotype 3)が同定され、54日目にピーク値を示した。HEVウイルス血症は潜伏期間を経て発現し、対数増殖後約50日前後にピークを示し、その直後にAST、ALT上昇と血中抗HEV抗体の出現を順に認めた。	8

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70107	2007/09/21	70537	G型肝炎	Epidemiol Mikrobiol Imunol 2006; 55: 136-139	チェコ共和国における静注免疫グロブリン(IVIG)投与患者の血清中におけるHGV陽性率を調査し、HGV陽性に関係したリスクを検討した。IVIG投与患者86例の内20例(23%)が、HGV RNA陽性であった。その内3例には肝機能検査値の緩やかな上昇が認められ、また1例は慢性リンパ性白血病であったが、IVIG投与前に診断されていた。IVIG投与患者のHGV感染率は高いが、肝疾患又はリンパ増殖のいずれの兆候とも関連していないと結論付けられる。	
70143	2007/10/26	70679	HIV	Clin Infect Dis 2007; 45: e68-e71	ボツワナで急性HIV-1感染スクリーニング中に特定された抗体陰性のHIV-1サブタイプC感染の初の症例を報告する。HIV-1抗体検査の結果は、迅速検査、通常の酵素免疫測定法及びウエスタンブロットで全て陰性であった。遺伝子組換えがないHIV-1サブタイプC感染は、ウイルスのgag、pol及びenv遺伝子のジェノタイピングによって確定された。臨床的に安定した状態からAIDS関連死までの期間は約3ヵ月だった。サブタイプCが優勢なアフリカ南部における血清学検査陰性HIV-1感染の調査の重要性が示された。	9
70165	2007/11/29	70768	HIV	Eurosurveillance 2007; 12(5): E070524.5 2007年5月24日	AIDS最新号において、LikataviciusらはEuroHIV surveillance network によるヨーロッパの供血血液のHIV陽性率についての14年間のモニタリングデータを提示した。この分析は、1990-2004年のWHO欧州地域のデータが網羅されている。2000-2004年の10万供血中の平均HIV陽性率は西欧1.7、中欧3.4、東欧36.7であった。1990年以降の変化では、西欧で低下、中欧で横ばい、東欧では急激な上昇が認められた。	10
70103	2007/09/21	70533	HIV	FDA/CBER 2007年5月23日	男性間性交渉者(MSM)からの供血に関するFDAの方針として、合衆国でAIDSの流行が始まった1977年以降は供血者として延期されている。MSMはHIV、HBVおよび他の感染のリスクが高いからである。米国赤十字によるとMSMのHIV有病率は一般集団の60倍、初回供血者の800倍、リピート供血者の8000倍高い。HIV検査は非常に正確であるが、HIVには感染後もHIVを検出できないwindow期がある。FDAは受血者を守るため、科学的なエビデンスが得られるまで、この方針を継続する。	
70112	2007/09/28	70553	HIV	Lancet 2007; 369: 621-623	2002年の国連レポートや米国国家情報会議は、中国には約100～200万人のHIV/AIDS患者がおり、感染爆発の危機が迫っているとしたが、2006年までの生存患者数は65万人と見積もられた。感染規模の過大な予測から、中国では様々な問題が生じた。HIV/AIDS対策に多大な予算を掛けたために、喫煙、結核など他の健康問題への対策が十分ではなかった。中国でのHIV/AIDS対策はハイリスク地域を中心に行うべきである。	
70112	2007/09/28	70553	HIV	Lancet 2007; 369: 623-625	2006年末までに台湾CDCに13702名のHIV-1/AIDS感染者が 報告された。2003年以降、HIV-1/AIDS感染生存者は急増し、台湾のHIV-1/AIDS感染者数は約3万人と推測され、台湾の感染率(2300万人中3万人; 1/767)は中国(13億人中65万人; 1/2000)よりも高い可能性が示された。リスク要因分析によると、静注薬物使用者の感染率は2005年には72.4%(2461/3399)であった。また垂直感染は2006年末までに19例が確定された。	

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70107	2007/09/21	70537	HIV	Vox Sanguinis 2007; 92: 113-120	20例の血友病患者が、1990年初頭以降、韓国で製造された血液凝固第IX因子の投与を受けてから1～2年後にHIV-1に感染していると診断された。血漿ドナーと血友病患者で検出されたウイルス間の遺伝子関連性を調べた結果、両者とも、HIV-1サブタイプBの韓国subcladeに感染していた。韓国で売血ドナーの血液から製造された凝固因子により、少なくとも20例の血友病患者がHIV-1サブタイプBに感染したことが明らかとなった。	
70151	2007/11/16	70737	HIV	第81回日本感染症学会総会・学術講演会 ポスターP26-1	これまで国内でのHIV-2感染症例はいずれの報告も外国籍患者であった。今回、日本人初のHIV-2感染例を経験した。77歳男性で、36年前セネガルで輸血歴がある。2006年6月、気管支喘息発作で入院となり、入院時HIVスクリーニング検査(ELISA)でHIV抗体高値となった。その後、Western Blot法による確認検査により、HIV-1抗体陰性HIV-2抗体陽性となった。遺伝子解析の結果、HIV-2サブタイプAに属し、セネガル株(60415K株)に最も近縁であった。	
70165	2007/11/29	70768	HIV感染、C型肝炎、B型肝炎	第31回日本血液事業学会総会 2007年10月 3-5日 シンポジウム4-2	日本赤十字社血液事業本部が関わる安全対策の取り組みと感染症リスクについて報告する。平成16年から18年までの3年間に全国の医療機関から日赤血液センターに報告された輸血関連感染症(疑い症例を含む)の報告数は749例であった。日赤の安全対策の実施によりHBV、HCV及びHIVの感染リスクは減少し、安全性は高くなった。しかし、HCV及びHIVも含め遡及調査の実施により確認された感染症例も少なくない。感染拡大を防止するための安全対策を引き続き講じていく必要がある。	11
70112	2007/09/28	70553	インフルエンザ	Science 2007; 315: 655-659	1918インフルエンザウイルスのヘマグルチニン受容体結合部位のごくわずかな変化により、ウイルスの伝播性が変化することが示された。2つのアミノ酸変異によって、ヒトのα-2,6シアル酸からトリのα-2,3シアル酸へと転換すると、フェレット間で呼吸器飛沫による感染を起こさないウイルスとなった。さらに、α-2,6およびα-2,3双方に特異性のある1918ウイルスは感染性が低かった。ヘマグルチニン受容体特異性が、哺乳類におけるインフルエンザ伝播に本質的な役割を果たす。	
70112	2007/09/28	70553	ウイルス感染	Canadian Blood Services 2006年12月18日	2006年12月18日付で、カナダ血液サービスは供血者が供血前に記入する供血記録の問診事項に一部修正を加える。カナダ保健局の指示により、ヒト以外の霊長類(サル、ヒヒ、チンパンジー、アカゲザル、あるいはその血液や唾液)との職業的接触に関する質問を追加した。サル泡沫状ウイルス(SFV)に関する認可された標準検査法がないため、供血者がこの質問に「はい」と答えた場合は無期限に供血延期となる。研究所で霊長類を扱う人、獣医師、動物園職員などが延期対象となるだろう。	
70143	2007/10/26	70679	ウイルス感染	CDC Press Release 2007年8月22日	米国疾病対策予防センター(CDC)と協力施設の科学者が、よく見られるアフリカフルーツコウモリの一種において、マールブルグウイルス感染を特定することに初めて成功した。マールブルグウイルスは、ヒトや霊長類に重篤で死に至ることも多い出血熱を引き起こす。コウモリがマールブルグウイルスを保有することが疑われていたが、証拠はなかった。この研究結果はPlos ONEに掲載された。この研究は、マールブルグウイルスの伝播についてより理解し、ヒトにおける感染拡大を予防・減少させる助力になると思われる。	12

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70165	2007/11/29	70768	ウイルス感染	J Clin Microbiol 2007; 45: 3008-3014	ヨーロッパでの出血熱は主にPuumalaウイルス(PUUV)またはDobravaウイルス感染による。ドイツ南東部Lower Bavariaでハンタウイルス感染患者31名について、酵素免疫測定法、免疫蛍光法、免疫ブロット法による診断を行った。標準的検査による抗体のPUUV特異的タイピングができない症例が2、3あった。3名の患者の急性期血清から得たPUUV RNAをRT-PCR を用いて増幅したところ、同地域で捕獲したハタネズミ から得たウイルス配列と非常に近縁であることが明らかとなった。	13
70107	2007/09/21	70537	ウイルス感染	Proc Natl Acad Sci 2007; 104: 11424-11429	マレーシアMelakaで高熱と急性呼吸器疾患に罹っていた39歳男性から未知のreovirusが分離され、Melaka virusと名づけられた。患者の家族も発症したが、彼らは発症前にコウモリと接触していた。遺伝子配列分析により、同ウイルスは同国Tioman島のフルーツコウモリから分離されたPulauウイルスと近縁であることが示された。同島住民は109例中14例(13%)が両ウイルスに陽性であった。Orthoreovirusがヒトの急性呼吸器疾患と関係があることが初めて示された。	14
70112	2007/09/28	70553	ウイルス感染	ProMED-mail 20070702.2108	2007年6月22日、ミクロネシアのヤップ保健局で集められた血液検体をCDCの研究所で検査した結果、ヤップでの最近の疾病はジカウイルスが原因らしいことが示された。ヤップのアウトブレイクは2007年4月に始まり、5月後半にピークに達し、現在も続いている。症状は斑点状丘疹、結膜炎、関節痛など軽症で、4-7日間続く。6月29日現在、42例がPCRとIgM分析によってジカウイルス感染と確定された。死亡例はない。	15
70112	2007/09/28	70553	ウイルス感染	ProMED-mail20070216.0586	西オーストラリア保健当局は、東Kimberleyと東Pilbara地区で蚊が媒介するウイルスの証拠が見つかったとして、西オーストラリア北部に居住あるいは滞在中の人々に、蚊に注意するよう呼びかけた。西オーストラリア大学が実施するサーベイランスプログラムによって、今年の雨期に初めてクンジンウイルスが確認された。クンジンウイルスは、蚊によって媒介されるウイルスで、マレーバレー脳炎(MVE)ウイルスと同じグループに属する。	
70112	2007/09/28	70553	ウイルス感染	ProMED-mail20070216.0596	ペルーの地方保健局長官は、Cuzco県La Convencion郡で黄熱による死亡例3例が発生したと報告した。Cuzcoの保健当局によると、このうち1例はMatoriato地区で発生したとのことである。当局は、La Convencion郡に向かう人全員を対象とした黄熱のワクチン接種キャンペーンを含む危機管理計画の策定を決定した。	
70107	2007/09/21	70537	ウイルス感染	ProMED-mail20070423.1325	オーストラリアのVictoriaで、一人のドナーから臓器移植を受けた3例が死亡したが、未知のウイルスが原因であった。このウイルスはﾘﾝﾊﾟ性脈絡髄膜炎ウイルスと近縁であったが、既存のスクリーニング法では検出されなかった。454 Life Sciencesによって確立された迅速シークエンシング技術とGreene Laboratoryによって開発されたバイオインフォマティクスアルゴリズムによって発見された。	

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70110	2007/09/27	70550	ウイルス感染	Transfusion 2007; 47: 162-170	輸血により、サルfoamyウイルス(SFV)感染が起こるかをアカゲザルを用いて調べた。感染ザルの血液を非感染ザルに輸血したところ、輸血されたサルの血液から8週後にプロウイルスDNAが検出され、その1週間後にセロコンバージョンが起こった。血しょう中に検出限界下限のSFVが検出された。また感染29週目に唾液中にSFVが検出された。輸血によりSFVが感染することが初めて示された。	
70143	2007/10/26	70679	ウイルス性脳炎	Neurology 2007; 69: 156-165	同種造血幹細胞移植(HSCT)後に急性大脳辺縁系脳炎を発症した患者9名の臨床、EEG、MRI、ならびに臨床検査特性を調べた。患者は、順行性健忘、不適切な抗利尿ホルモン分泌症候群、軽度CSF多球症、一時的なEEG異常を特徴とした。MRIでは、T2、FLAIR、DWI画像にて、鈎、扁桃体、内側嗅領、海馬領域内に高信号域を認めた。PCRを用いた初回腰椎穿刺CSFの検査では9名中6名がHHV6陽性であり、同脳炎はHHV6と関連がある可能性が示唆された。	16 (=6)
70103	2007/09/21	70533	ウエストナイルウイルス	CDC/MMWR 2007; 56(4): 76-79	ID-NATを用いた強化スクリーニング開始以降に、初めて西ナイルウイルス輸血感染症例が報告された。2006年に免疫不全患者2例が、感染ドナー1例(献血時のMP-NATの結果は陰性)由来の血液製品を投与された後、西ナイル神経侵襲性疾患を発症した。今回の例はID-NATは実施されておらず、ID-NATトリガーを促進することが重要である。	
70107	2007/09/21	70537	ウエストナイルウイルス	The New York Times 2007年7月26日	米国におけるウエストナイルウイルス症例数は1年前の約4倍であり、大流行がおこる可能性があると政府研究者が報告している。昨年は米国で4,269症例が報告され、この中には1,495例の脳症が含まれ、177人が死亡した。今年はこれまで122症例が報告され、カリフォルニア州と南北ダコタ州で最も多いが、昨年の同時期は33例のみであった。今年は既に脳症が42例および死亡が3例ある。	17
70143	2007/10/26	70679	ウエストナイルウイルス	第144回日本獣医学会学術集会 2007年9月2-4日	ウエストナイルウイルスは、近い将来、日本にも侵入する可能性があるため、日本産蚊の室内継代株を用いて増殖・媒介能を調べた。アカイエカ、ヒトスジシマカ、オオクロヤブカでウイルス注入実験を、アカイエカ、ヒトスジシマカで吸血実験をしたところ、全種類の蚊においてウイルスの増殖が観察された。媒介試験では、アカイエカ注入、吸血両群、ヒトスジシマカ2系統の注入群、1系統の吸血群では供試したすべてのマウスが12日以内に死亡し、死亡したマウスからはWNV が検出された。	18
70107	2007/09/21	70537	クロストリジウム感染	YOMIURI ONLINE (2007年2月22日 読売新聞)	千葉県の船橋市立医療センターは22日、同県内の50歳代の男性が、主に牛の病気の原因とされる「気腫疽菌」に感染し、死亡したことを明らかにした。人への感染が報告されたのは世界初である。気腫疽菌は傷口などから動物の体内に入り、筋肉が壊死する「気腫疽」を発症させる。同センターは、「気腫疽菌は人には感染しないというのがこれまでの常識だった。詳しい感染経路を調べることが今後の課題」としている。	
70101	2007/09/19	70492	クロストリジウム感染	イザ(産経新聞) 2007年2月23日	千葉県の船橋市立医療センターは22日、2006年2月に搬送され、死亡した同県内の50歳代の男性から気腫疽菌が検出されたと2007年2月22日に発表した。人への感染が報告されたのは初めてである。	

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70165	2007/11/29	70768	チクングニヤウイルス感染	Pediatr Infect Dis J 2007; 26: 811-815	チクングニヤウイルス感染が大流行したレユニオン島の5つの新生児医療部門で同ウイルスの母子感染を調べるため、後ろ向き記述的研究を実施した。母親は出産時に徴候があったか又は新生児が出生初日に発病したかをスクリーニングし、新生児38名を登録した。無症候の2名を除き、全母親が周産期(分娩4日前～1日後)に症状があった。全新生児が発熱(79%)、疼痛(100%)などの症状を示し、脳脊髄液のPCR法は24名中22名で陽性であった。高い罹患率の周産期母子伝播の可能性が初めて示された。	19
70112	2007/09/28	70553	チクングニヤウイルス感染	Emerg Infect Dis 2007; 13: 147-149	最近マレーシアでは、7年間検出されていなかったチクングニヤウイルス感染が再興した。分離ウイルスのゲノム配列は、1998年のアウトブレイク時のMalaysian 分離ウイルスの配列との相同性が高かった。この感染の再興は、他のインド洋諸国における流行とは関係ないが、マレーシア特有のチクングニヤが流行する可能性が浮上している。	
70165	2007/11/29	70768	チクングニヤウイルス感染	Eurosurveillance 2007; 12(9): E070906.1	チクングニヤ熱は2005年以来、大規模な流行がインド洋諸島とインドから報告されているが、これまでヨーロッパ地域内での蚊による感染伝播は発生していなかった。2007年8月にイタリアのエミリア・ロマーニャ州ラヴェンナ県衛生当局は異常に多数の発熱患者発生を検知し、臨床・疫学調査を行った。血清学的検査およびPCR法でチクングニヤ熱と確定された。更にヒトスジシマカからもPCR法によりチクングニヤウイルスが確認された。2007年9月4日までに合計197名の患者が報告されている。	20
70112	2007/09/28	70553	デング熱	ABC Newsletter 2007年7月20日	オーストラリアのクイーンズランド州北部におけるデング熱アウトブレイクにより、赤十字血液サービスは流行地に滞在した人から供血された血液製剤の廃棄を余儀なくされた。局地的アウトブレイクは3月末にSouth Townsvilleで始まったが、5月14日以降、新規症例は記録されていない。赤十字のスポークスマンによると、供血以前に同地を訪れた供血者由来の赤血球・血小板は破棄するが、血漿は使用できるとのことである。供血制限は、アウトブレイクの終息が正式に宣言されるまで継続される。	21
70117	2007/10/05	70580	デング熱	CDC/MMWR 2007; 56(31): 785-789	2005年7月にデング出血熱(DHF)症例1例がTexas州Brownsvilleの住民において報告された。2005年8月に隣接するメキシコTamaulipas州の保健当局はデング熱症例1251例が発生しているデング熱のアウトブレイクを報告し、内223例(17.8%)がDHFであった。臨床的および疫学的調査の結果、同地域でのデング熱アウトブレイクに伴うDHF症例の割合は、2000－2004年はデング熱症例541例中20例(3.7%)であり、増加していることが明らかとなった。	22
70107	2007/09/21	70537	デング熱	Trans R Soc Trop Med Hyg 2007; 101: 738-739	日本人のデング熱患者(28歳、女性)の血漿サンプル中ではなく尿及び唾液中でデングウイルスを検出することに成功した。発症後7、14および25日目の血漿検体中で抗デングウイルス抗体は同定されたが、デングウイルス遺伝子は検出されなかった。発症後7、8および14日目の尿、ならびに7日目の唾液からデングウイルス1型遺伝子が検出された。現在の研究の結果は、尿及び唾液中のデングウイルス遺伝子の検出が有効な診断方法、特にウイルス性出血の子供の診断方法になりうることを示唆している。	23

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70165	2007/11/29	70768	トリパノソーマ症	ABC Newsletter 2007年9月14日	AABBはCDCからAABBシャーガス病バイオビジランスネットワーク強化をするための資金を受けている。2007年9月13日現在、710名の反復反応性供血者がT. Cruziに対する抗体の追加RIPA試験を行った結果、196名がRIPA陽性、486名が無反応で、残りは結果がまだ出ていない。13の検査所がシャーガスネットワークにデータを報告し、18の検査所が同ネットワークにアクセスしている。	24
70143	2007/10/26	70679	トリパノソーマ症	CMAJ 2007;177: 242	カナダ血液サービスは、2008年後半の血液製剤製造プロセス見直しの際に北緯49度以北では稀にしか見られないシャーガス病のスクリーニングを開始する。2種類のシャーガス病検査法がカナダ保健省の認可を待っている。供血血液の検査実施は、血小板製剤の製造を「バフィーコート」法に切替えてからとなる。メキシコや中南米では800万人～1,100万人がシャーガス病の保因者であり、毎年45,000人以上死亡している。カナダでは、これまでに輸血による感染が2例マニトバ州で発生した。	25
70112	2007/09/28	70553	トリパノソーマ症	Reuters AlertNet 2007年4月13日	WHOによると、感染の数十年後に死亡する可能性もある寄生虫症、シャーガス病が、不適切な血液スクリーニングが原因でラテンアメリカから米国やヨーロッパに拡大している。WHOはバイエル社の支援を受けて、今や「地球規模の問題」となったシャーガス病根絶のための事業を拡大している。シャーガス病に感染している人は900万人にのぼると見られ、その多くはラテンアメリカの農村部の子どもである。最近では大規模な移民の影響で米国、スペインや他の欧州諸国に広がっている。	
70112	2007/09/28	70553	トリパノソーマ症	Transfusion 2007; 47: 540-544	神経芽細胞腫(ステージ4)を発症した3歳半の女児が複数の血液成分製剤投与を受けた後、Trypanosoma cruziによるシャーガス病と診断された。輸血された製剤の全供血者の血液を再検査したところ、初回供血者1名がT. cruzi抗体陽性であることが判明した。当該供血者は、ボリビア出身であり、17年前に米国に移住した。移住後は母国に帰国していない。本症例は、米国・カナダでの輸血によるシャーガス病感染の7例目の報告である。シャーガス病スクリーニング検査が必要であることを示している。	
70158	2007/11/21	70746	パルボウィルス	J Gen Virol 2007; 88: 2162-2167	ヒト血漿プール中に新規のパルボウイルスPARV4とその変異株であるPARV5が存在することが最近示された。4株のPARV4と2株のPARV5のDNA配列を分析したところ、PARV5はPARV4と同様に2つのオープンリーディングフレームを持ち、PARV4とPARV5は92%近くのヌクレオチド相同性を示した。両者は密接な関係のあるジェノタイプであり、ジェノタイプ1と2(PRV5と呼ばれていたもの)から成るPARV4という1つのウイルス名を使用することを提案する。	26
70107	2007/09/21	70537	パルボウィルス	Transfusion 2007; 47: 883-889	1993-1998年及び2001-2004年の間に製造された6つの第Ⅷ因子濃縮剤の284ロットについて、in-house NAT法によりパルボウイルスB19 DNAを測定し、抗B19 IgGも併せて測定した。その結果、B19 NAT非スクリーニング血漿から調製した製剤のB19 DNAの陽性率及びレベルは高かったが、製造方法が異なると、製品間で様々であった。血漿のB19 NATスクリーニングは、最終製品中のB19 DNAレベルを下げ、大半の例で検出限界以下とさせ、B19伝播のリスクを減少させた可能性がある。	

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70107	2007/09/21	70537	ヒトポリオーマウイルス感染	J Virol 2007; 81: 4130-4136	ヒトの気道からの検体をウイルススクリーニングし、KIポリオーマウイルスと暫定的に名付けた未知のポリオーマウイルスを同定した。このウイルスは、遺伝子のearly領域では、他の霊長類のポリオーマウイルスに系統遺伝学的に近縁であるが、late領域では、既知のポリオーマウイルスに対して相同性が少ない(アミノ酸同一性30%未満)。このウイルスは、PCRによって、鼻咽頭吸引物637例中6例(1%)と便検体192例中1例(0.5%)で検出されたが、尿及び血液検体では検出されなかった。	
70107	2007/09/21	70537	ヒトポリオーマウイルス感染	PLoS Pathogens 2007; 3: 595-604	急性呼吸器感染症に罹った患者からの呼吸分泌物中に存在する新規のポリオーマウイルスを同定し、WUウイルスと名付けた。WUウイルス遺伝子は5229bpで、Polyomaviridaeファミリーの特徴を持つ。系統遺伝学的分析から、このWUウイルスは、既知の全てのポリオーマウイルスとは異なっていることが明白となった。オーストラリア及び米国の急性呼吸器感染症患者2135例中43例からWUウイルスが検出され、地理的に広く分布していることが示唆された。	
70112	2007/09/28	70553	マラリア	ABC Newsletter 2007年7月6日	FDAは、初めて認証された米国のマラリア用迅速テスト、Binax NOWマラリア検査の使用を許可した。同検査は、非常に迅速で使用が簡便で、全血検体をディップスティックに2、3滴つけて15分後には結果が得られる。検査結果の確定には標準的顕微鏡検査法を用いなければならない。米国外のマラリア流行地域で行った多施設試験において、標準的顕微鏡診断と比較して当該検査の正確度は95%であった。	27
70112	2007/09/28	70553	マラリア	ProMED-mail20070501.1414	ジャマイカ保健省によると、2007年4月の1ヶ月間に新規のマラリア症例11例が報告された。内2例はメスのハマダラカが媒介する熱帯熱マラリア原虫によるものであった。また、2006年12月に最初の症例が報告されて以降、輸入感染症例が7例あった。2007年4月1～21日の間に実施された884検体の検査の結果、血液検体陽性率は0.7～1.8%で減少を続けている。最近、Anopheles albimanus蚊がマラチオン殺虫剤に耐性を示し始めたことが確認されたため、感染拡大を防ぐために代わりの殺虫剤を探している。	
70112	2007/09/28	70553	マラリア	Vox Sanguinis 2007; 93(Suppl.1): P239	韓国における輸血によるマラリア感染の発生について調査した。マラリア診断前6ヶ月間以内に供血を行った供血者は、2005年5月～2006年8月の三日熱マラリア患者2056名中46名(2.2%)であった。46名の保管血液51検体についてPCRを実施し、PCR陽性の血液成分を輸血された全受血者を調査したところ、1名の受血者に輸血によるマラリア伝播が確認された。PCRに基づくマラリア遡及調査は、輸血によるマラリア伝播の特定に役立つ。	28
70143	2007/10/26	70679	リケッチア症	Jpn J Infect Dis 2007; 60: 241-243	血清学的、微生物学的に確定された日本紅斑熱の初めての死亡症例を報告する。淡路島在住の77歳男性で、2005年9月2日に食欲低下を呈し、翌日、下腿に皮疹が出現、4日目に38.7℃の高熱、歩行障害、構音障害が出現、肝機能障害が急速に進行し、DIC、消化管出血により8日目に死亡した。右肩にダニ刺し口があった。血液よりDNAを抽出し、PCRを実施したところ、塩基配列はR. japonicaと100%一致した。日本紅斑熱は増加傾向にあり、注意が必要である。	29

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70143	2007/10/26	70679	リケッチア症	朝鮮日報 2007年8月21日	韓国では最近ツツガムシ病の患者が急増している。2007年8月20日、疾病管理本部の発表によると、2002年に1,919人だったツツガムシ病の患者数が、04年は4,698人、06年には6,420人に増加したことが分かった。1993年末に法定伝染病に指定されて以来、患者数は実に25倍以上増加した。ツツガムシ病は、主に9月以降、ツツガムシ菌に感染したツツガムシ(ダニの一種)の幼虫に刺されることにより感染する。10日間程度の潜伏期を経ると、突然高熱が発生し、目の充血、頭痛、筋肉痛、発疹などの症状が現れる。	30
70107	2007/09/21	70537	レンサ球菌感染	ProMED-mail20070223.0668	米国の科学者は北アメリカで初めて報告されたStreptococcus suis髄膜炎のヒト感染例を確認した。健康であった59歳の男性農業従事者が髄膜炎で入院し、S. suis感染と判明した。S. suisはブタで重病を起こすグラム陽性球菌であり、ブタを扱う職業の人は注意が必要である。保健当局はヒトからヒトへの感染のおそれはないとしている。	
70112	2007/09/28	70553	BSE	ProMED-mail20070302.0734	ニュージーランド食品安全局はBSEを取り巻く最新の科学と実際の知識を踏まえて、ウシ及びウシ加工品の輸入規制を改訂する方針である。新しい規制は科学的証拠や最近の国際的な規制に合致したものとするため、輸出する国のBSEリスクステータスの分類に、国際的に認められた3カテゴリーシステムを導入する。ゼラチンは、原材料の起源およびBSEリスクのある国からの輸入を問わず、全てのゼラチンの売買が自由化される。	
70112	2007/09/28	70553	クロイツフェルト・ヤコブ病	Emerg Infect Dis 2007; 13: 162-164	1999年4月から2005年3月まで日本のCJDサーベイランス委員会に登録されていたプリオン病患者について分析した。日本のプリオン疾患患者597名のうち11名(1.8%)が、発症の前後1ヶ月以内に眼科手術を受けた。眼科医はいずれもプリオンタンパクの感染性を除去するには不十分な滅菌しか行われていない手術器具を再使用していた。眼科医は、プリオン疾患が眼症状を引き起こす可能性があることを認識し、可能な限り使い捨て器具を使用すべきである。	
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	ABC Newsletter 2007年2月9日 7-8ページ	将来のvCJDによる死亡率は、供血に関する公衆衛生上の施策によって予想されていたよりも遙かに低くなるだろうと英国の研究者が報告した。Royal Society Journal Interface誌オンライン版によると、2080年までの輸血によるvCJDの死亡例は50例と予測される。感染牛の摂食によるvCJD感染が排除されたため、現在では輸血による伝播が最も可能性が高いと研究者は話している。	
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	ABC Newsletter 2007年5月4日	イスラエルで血液事業を行っているMagen David Adomは、変異型クロイツフェルト・ヤコブ病(vCJD)に関する供血延期基準を変更し、1980年以降にフランス居住歴がある人の供血を可能とした。1980年から10年間のうちにイギリス、アイルランド、ポルトガルに居住歴のある人は、引き続き供血禁止となる。また、輸血を受けた人、B型肝炎やC型肝炎患者と一緒に住んでいた人、入れ墨を入れた人、内視鏡検査を受けた人、未検査の動物に噛まれた人の供血延期期間を短縮した。	

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70125	2007/10/24	70644	異型クロイツフェルト・ヤコブ病	Biochim Biophys Acta 2007; 1772: 598-609	変異型クロイツフェルト・ヤコブ病(vCJD)がウシのBSEと同じプリオン株によってヒトに発症するという認識はヒトプリオン病の分子生物学に関する正確な理解の必要性を注目させた。多数のプリオン病患者から得られた詳細な臨床的、病理学的および分子学的データはヒトプリオン病における表現型の多様性が、疾病に関連したPrPアイソフォームの伝播に部分的に関係していることを示した。ヒトにおけるプリオン感染の潜伏期間は50年を越えることもあるので、ヒトvCJD流行の程度を予測するにはまだ数年要する。	31
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	Biologicals 2007; 35: 79-97	ドイツにおいて、vCJDが血液供給へ及ぼす影響について実際の集団データを基にモデル計算を行ったところ、輸血を介した伝播がvCJDを永続化するような可能性はなかった。更に、受血経験者を供血から排除しても輸血の安全性向上にはほとんど寄与しないが、血液供給には多大な影響を及ぼすと考えられた。そのためドイツにおいては受血経験者の除外は推薦されなかった。	
70151	2007/11/16	70737	異型クロイツフェルト・ヤコブ病	Biologicals 2007; doi:10.1016/j.biologicals.2007.04.005	異なるポアサイズのウイルス除去膜を使用し、異なる処理を行ったスクレーピープリオン蛋白(PrPSc)の除去能力を評価した。超音波処理により粒子径分布を至適化するように調製した263K MFをスパイク物質として使用したときは、75nmのろ液中にPrPScが検出された。15nmのろ過のみが全ての条件でウエスタンブロット法の検出限界以下までPrPScが除去されることが示された。しかし、 1条件下の15nmろ液のバイオアッセイの結果では、感染性PrPScが確認された。	32
70103	2007/09/21	70533	異型クロイツフェルト・ヤコブ病	Curr Opin Hematol 2007; 14: 210-214	赤血球製剤の輸血によるヒトでのvCJD感染症例が報告されている。げっ歯類のTSEに関する実験で、赤血球製剤の感染性は赤血球自体に関係があるのではなく、残存している白血球や血漿のような製剤中の他の成分に関係することが示された。vCJD因子がヒト赤血球と結合できないことが示されたら、vCJDが発生している国の血液サービスは輸血前に洗浄や濾過により感染性のある液相を取り除くことが賢明かもしれない。	
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	Emerg Infect Dis 2007; 13: 89-96	vCJD二次感染防止のため、輸血歴のある人の供血を禁止している国もある。Dynamic age-structured modelを用いて、この措置の効果を検討した。これは、供血者の行動、CJDの症例対照試験、受血者の年齢分布および受血者の死亡の疫学的データに基づくモデルとしては初めてのものである。食品によりヒトに導入されたvCJDの様な感染は、輸血のみにより拡大する可能性はないこと、また、輸血歴のある人を供血から除外することにより感染を免れるのは1%未満の症例にすぎないことが予測された。	

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70114	2007/09/28	70555	異型クロイツフェルト・ヤコブ病	FDA/CBER 2006年11月27日	FDAは、米国で認可されたヒト血漿由来第VIII凝固因子製剤(pdFVIII)の使用に係る潜在的vCJDリスク評価草案を作成した。FDAの評価モデルの結果は、血友病Aおよびフォンウィルブランド病患者に使用されるpdFVIII製剤の、vCJD感染リスクは非常に低いが、ゼロではないかもしれないことを示唆した。製造工程での原因物質除去レベルにより、vCJD感染リスクは大きく変動する。	
70107	2007/09/21	70537	異型クロイツフェルト・ヤコブ病	FDA/CBER 2006年11月7日	英国血漿由来の第XI因子製剤が、1989-2000年に米国で50名以下の患者に使用されたと推定される。モデルを用いたリスク評価の結果、1998年まで第XI因子製剤を製造するために使用された血漿プールの1.6%～50%がvCJD病原体を含んでいる可能性があった。しかし、これまで血漿由来製剤の投与を受けた患者において、世界中で一件もvCJDの症例は報告されていない。製造工程におけるvCJD除去、使用量、曝露経路および英国ドナーのvCJD有病率がリスクに影響を与える重要な因子である。	33
70103	2007/09/21	70533	異型クロイツフェルト・ヤコブ病	FDA/CBER 2007年3月15日	FDA、CDCおよびNIHを含む米国Public Health Serviceは、米国で承認された血漿由来第VIII因子製品を投与された血友病Aおよびフォンウィルブランド病患者のvCJDリスクは極めて小さいとの見解を示した。血漿由来第IX因子を含む他の血漿由来製品によるvCJDリスクは同程度もしくは更に小さいと思われる。	
70125	2007/10/24	70644	異型クロイツフェルト・ヤコブ病	FDA/CBER 2007年3月15日	近年，米国で承認されている第8因子，第9因子を含む血漿由来の血液凝固因子および免疫グロブリンやアルブミンのような他の血漿由来製品のレシピエントにおけるvCJDリスクに関する懸念が挙がっている。これに対し，米FDAはリスク評価を行った。この評価に基づいて，US Public Health Serviceは米国で承認されている第8因子を投与されている患者のvCJDリスクは非常に低く、その他の血漿由来製品(第9因子を含む)のvCJDリスクは同程度か更に低いとしている。	34
70103	2007/09/21	70533	異型クロイツフェルト・ヤコブ病	FDA/CBER 2007年5月30日	近年、英国で得られた血漿から作られた血漿第XI因子(pdFXI)を投与された患者でのvCJDリスクが関心を集めている。1989年から2000年の間に米国では約50人に英国血漿由来のpdFXIが投与された。世界中でこれまで血友病や他の凝血疾患の患者においてvCJDは全く報告されていない。これらの患者は長期間にわたり血漿由来製剤を大量に投与されていることから、pdFXIを投与された患者でのvCJDリスクは小さいと考えられる。	
70107	2007/09/21	70537	異型クロイツフェルト・ヤコブ病	J R Soc Interface doi:10.1098/rsif.2007.0216 Published online	血液由来のvCJDの流行の大きさを探るために感度分析を行い、公衆衛生的介入の有効性について調査した。数学的モデルを開発し、悲観的モデリング仮定で評価すると、自己持続的流行が起こるならば2080年までに900例以内、楽観的仮定では250例以内となった。大規模な又は自己持続性流行に至るシナリオの可能性はあるが実現性は低く、輸血を受けたヒトからのドネーション禁止措置等の公衆衛生的介入が有効である。	

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70128	2007/10/24	70647	異型クロイツフェルト・ヤコブ病	J Vet Diagn Invest 2007; 19: 142-154	米国で牛海綿状脳症(BSE)と診断されたウシ2例に関する報告である。症例1では脳幹の閂領域に海綿状変性及びPrPScの沈着がみられたが、症例2ではPrPScは検出されたが、明らかな空胞状の変化はみられなかった。ウェスタンブロット法で、症例1は典型的なBSE分離株と似た分子的特徴を示したが、症例2のPrPScは高分子量に位置する異常な電気泳動パターンを示した。両症例のプリオン蛋白遺伝子の配列を決定したところ，ウシについて過去に報告された配列の多様性の範囲内であることが示された。	35
70128	2007/10/24	70647	異型クロイツフェルト・ヤコブ病	J Virol 2007; 81: 4305-4314	無細胞PrP変換法を用いてシカの慢性消耗性疾患(CWD)分離株に対するげっ歯の感受性を調べたところ、一部のげっ歯類が感受性を有することが示唆された。これらの結果はCWD罹患シカの脳組織をげっ歯類の脳内に接種する実験でも確認された。シリアンゴールデンハムスターにおけるCWD分離株の継代では、平均潜伏期間が異なり、明らかな神経病理学的パターンを示す分離株を特定した。CWDは一部のげっ歯類に伝播し、少なくとも2種類のTSE分離株が存在する可能性が示唆された。	36
70128	2007/10/24	70647	異型クロイツフェルト・ヤコブ病	PLoS ONE 2007; 5: e435	ハムスター順応性263Kスクレイピープリオンで土壌を汚染し、土壌中PrPScの存在をウエスタンブロットで、生物学的活性および感染性をハムスターバイオアッセイで分析した。同プリオンは少なくとも29ヶ月以上土壌中に存在し、汚染土壌または土壌の水性抽出液を摂餌したシリアンハムスターにおいてスクレイピーが誘発された。また土壌中PrPSc検出にはPMCA (protein misfolding cyclic amplification)反応が利用できることが示唆された。	37
70107	2007/09/21	70537	異型クロイツフェルト・ヤコブ病	PLoS Pathogens 2007; 3: 659-667	経口的又は非経口的にスクレイピーを投与したハムスターの皮膚にPrPScが沈着するかを調べた。経口摂取したハムスターでは発症前にPrPScが検出され、発症時にはPrPScの蓄積がみられた。PrPScは皮膚の角化細胞ではなく神経線維に局在し、皮膚におけるPrPScの沈着は感染経路やリンパ組織感染に依存しなかった。神経が介在する遠心的な皮膚へのプリオン拡大が示された。更に、スクレイピーに自然感染したヒツジを調べたところ、5頭中2頭の皮膚検体中にPrPScが検出された。	38
70165	2007/11/29	70768	異型クロイツフェルト・ヤコブ病	Prion 2007; 2007年9月26-28日 Edinburgh P04.51	73歳の受血者で生前に特定されたvCJDの非典型的症状の報告である。患者は1997年12月に輸血を受けたが、供血後にvCJDを発症した供血者由来の赤血球製剤であった。輸血から6年後、受血者は疲労及び集中困難を訴えたが、神経学的検査及び脳MRIは正常であった。この6ヵ月後に神経学的症状が発現し、進行したが、血清学的検査は正常であった。MRIでは視床背側核全体の顕著な信号変化が示された。vCJDの長期潜伏期間と無症候状態は、重大な公衆衛生問題を提示する。	39
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	Proc Natl Acad Sci 2007; 104: 10998-11001	アミロイドを含有するフォアグラにアミロイド促進因子(AEF)活性があるかを調べた。市販のフォアグラから抽出したアミロイドA蛋白含有フィブリルを、二次性アミロイドーシスを起こすトランスジェニックマウス9匹に静脈内投与したところ全例で、また経口投与した場合は8匹中5匹でアミロイドの組織沈着が見られた。一方、対照群では全く組織沈着は見られなかった。加熱によりフォアグラのAEF活性は弱まったが、消失しなかった。アミロイドーシスは伝播性で、プリオン関連疾患の感染性と類似する可能性がある。	40

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70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	Proc Natl Acad Sci USA 2007; 104: 1965-1970	スクレイピー22L株に感染した神経芽細胞腫細胞およびFUクロイツフェルトヤコブ病病原体に感染した視床下部GT細胞は直交配列で高密度な25nmウイルス様粒子を示した。この粒子は膜に囲まれた不完全結晶で、A型レトロウイルス粒子クラスターや異常PrP原線維とは別に存在し、形態学的にも異なっていた。またPrP抗体でラベルされず、ホルボールエステル処理で増加しなかったことから、プリオンではなかった。この粒子は後期PrP脳病変を誘発するTSE原因ビリオンである可能性がある。	
70128	2007/10/24	70647	異型クロイツフェルト・ヤコブ病	Proc R Soc B 2007; 274: 1497-1503	英国人消費者のBSE感染したヒツジ肉への理論的曝露を評価し、屠殺場での制御により達成されうるリスク軽減を見積もる数学モデルを構築した。その結果、1頭の感染ヒツジが感染ウシ1頭に比べ10～1000倍の感染物質をもたらすと予測された。また、英国でBSE感染が続いているヒツジの群はわずか4群と95%の信頼度で推定した。組織検査に基づいた戦略、12ヶ月齢制限および危険部位の拡大よりも、6ヶ月齢制限および遺伝子型に基づいた戦略の方が感染リスク軽減に更に有効と考えられた。	41
70112	2007/09/28	70553	異型クロイツフェルト・ヤコブ病	SEAC/Position Statement 2007年6月13日	英国保健省はSEACに歯科治療処置を介したvCJD伝播のリスク概算を目的とした初期研究の知見についての助言を求めた。初期研究では、歯科処置によるvCJD伝播のリスクが予想より高いことが示唆された。ガイダンスは今年初め歯髄治療用器具の使い捨てを勧告した。公衆衛生上の影響についてのより綿密な考察と、さらなるリスク減少手段の特定のため、全ての歯科治療のリスクについて詳細で包括的な評価を早急に行うことも重要である。	42
70125	2007/10/24	70644	異型クロイツフェルト・ヤコブ病	Transfus Clin Biol 2006; 13: 312-316	vCJDが輸血により伝播するエビデンスがあるかを調べるために1997年に英国で試験が開始された。今までのところ本試験により、輸血によるvCJD伝播と思われる症例が3例特定された。2例は臨床症状を呈し、もう1例は臨床症状発現前の症例である。	43
70103	2007/09/21	70533	異型クロイツフェルト・ヤコブ病	Transfus Clin Biol 2006; 13: 320-328	血漿製品によるプリオン感染症例は今まで見られていない。国によって対策は異なるが、vCJDやBSEのある国での疫学的調査、特定の期間にBSE発生国へ旅行したり、住んでいた人や輸血や組織移植を受けた人に対する供血延期措置、血漿中の白血球除去、複雑な産業的分画過程中でのプリオンの除去などが行われている。エタノール分画、デプスフィルトレーションおよびクロマトグラフィーは数logのプリオンを除去できる。またナノフィルトレーションもプリオン除去に有用な方法である。	
70125	2007/10/24	70644	異型クロイツフェルト・ヤコブ病	Transfusion 2007; 47: 1418-1425	PrPTSEのmisfolded protein diagnostic (MPD)アッセイは、TSE感染マウス、正常マウスおよびPrPノックアウトマウス由来の脳組織中で、ウェスタンブロットシグナルと相関し、異なったペプチド配列を持つ試薬ではnegativeアッセイシグナルであった。血漿又は血清に対して適用した場合には、MPDアッセイは未感染の対照と比較して、種々の実験的および自然TSE感染由来検体を区別した。MPDアッセイは、ヒト及び動物のプリオン病の前臨床及び臨床診断にあたり有用であると考えられる。	44

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70107	2007/09/21	70537	肝炎	Med Mol Morphol 2007; 40: 23-28	ALTが高く、HCV抗体とB型肝炎表面抗原が陰性である供血者からの血漿検体中のウイルス様粒子(VLPs)を視覚的に捉えようと試み、また、このVLPsと非経口的に感染するGBV-C/HGVの遺伝子との関係を調べた。その結果、循環血液中のVLPsの検出率は、有意にALTレベル上昇と関係(P<0.001)していたが、VLPsを含む血漿のいずれにも、GBV-C/HGV RNAは検出されなかった。電子顕微鏡で球状のVLPsが確認され、それらが非B非C型肝炎に関係していることが示唆された。	
70130	2007/10/24	70649	寄生虫感染	Int J Med Microbiol 2007; 297: 197-204	ドイツにおけるヒトバベシア症の初めての症例を報告する。患者は結節性リンパ球性ホジキンリンパ腫が再発し、脾臓摘出されたドイツ人の63歳男性で、リツキシマブ投与後、貧血とヘモグロビン尿による暗色尿のため入院した。末梢血塗抹標本で梨状の寄生虫赤血球封入体が確認されバベシア症と推定され、Babesia特異的18S rDNA PCRによって確認された。シークエンス分析によりEU1と99.7%の相同性があり、EU3と名づけられた。寄生虫が消えるまでにはatovaquoneによる長期治療を要した。	45
70112	2007/09/28	70553	結核	Emerg Infect Dis 2007; 13: 380-387	第二選択抗結核剤6クラスのうち3つ以上に耐性を示す多剤耐性結核を広範囲薬剤耐性結核(XDR TB)と定義し、2000年～2004年のSupranational Reference Laboratoriesのネットワークを調査した。48カ国からのMycobacterium tuberculosis分離株17,690のデータが提供され、多剤耐性分離株3,520のうち、347(9.9%)がXDR TBであった。	46
70112	2007/09/28	70553	細菌感染	ABC Newsletter 2007年4月13日 21ページ	2004年度から2006年度にかけて米国食品医薬品局(FDA)に報告された輸血関連副作用による死亡症例数である。3年間の合計は219例で、内訳はTRALI86例(39.3%)、その他の副作用(ABO不適合以外の溶血反応、輸血関連心過負荷、細菌感染、アナフィラキシーなど)67例(30.6%)、細菌感染20例(9.1%)、ABO不適合による溶血反応15例(6.8%)、輸血が原因である可能性が否定できない症例31例(14.2%)となっている。	
70112	2007/09/28	70553	細菌感染	American Society for Microbiology 107th Annual Meeting; L-004 2007年5月21－25日	日本の三次医療施設である自治医科大学病院(病床数1082床)において、2006年4月1日～8月31日に、患者28名の血液培養からBacillus cereusが検出された。リネン類の汚染と末梢静脈ラインの不適切な取り扱いが原因であると考えられた。一時的にリネン類のオートクレーブ処理を行い、洗濯機を洗浄し、末梢静脈ライン管理について職員の教育を行ったことで、B. cereus陽性血液培養はその後検出されなかった。	
70112	2007/09/28	70553	細菌感染	Clin Infect Dis 2007; 44: 1408-1414	2005年3月、米国ネブラスカ州の病院で複数の病室において、無針静注カテーテルコネクターバルブが導入された時期に血流感染の急激な増加が見られた。一次血流感染について調査を行ったところ、一次血流感染と無針静注カテーテルコネクターバルブの使用との間に有意な関連性が認められた。細菌培養を行った37個のバルブのうち24.3%から微生物が検出され、主にコアグラーゼ陰性ブドウ球菌であった。無針コネクターバルブの評価には市場導入前に感染リスクの査定を含めるべきである。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70112	2007/09/28	70553	細菌感染	Transfusion 2007; 47: 1134-1142	アメリカ赤十字で2004年3月1日～2006年5月31日の期間に1,004,206例の供血で細菌培養検査が行われ、その内186例が陽性であった。関連するアフェレーシス血小板293製剤のうち1件を除くすべての輸血が回避された。両腕法を用いて採取した場合の細菌培養陽性率は、片腕法と比較して有意に高かった。また、スクリーニング陰性の製剤に関係した敗血症性輸血反応が20例(うち死亡3例)報告されたが、両腕法を用いて採取した場合の頻度は片腕法と比較して4.7倍であった。	47
70107	2007/09/21	70537	鳥インフルエンザ	Emerg Infect Dis 2007; 13: 1081-1083	高病原性鳥インフルエンザウイルス(H5N1)を含むインフルエンザウイルスが、血液安全性の脅威となるおそれがある。ミニプール核酸増幅法を用いて10,272例の血液ドナー検体を分析した。この検査法の測定感度は、一般的インフルエンザウイルス用プライマーについては804 geq/ml、インフルエンザ(H5N1)サブタイプ特異的プライマーでは444 geq/mlであった。インフルエンザウイルスに対して、このようなスクリーニング検査が可能であることが示された。	48
70162	2007/11/21	70750	鳥インフルエンザ	Emerg Infect Dis 2007; 13: 1348-1353	2006年5月にインドネシアのスマトラ北部でおよび2005年12月にトルコ東部の家族で観察されたトリインフルエンザH5N1の集団が、ヒト-ヒト伝播によるか否かを統計的方法を用いて調べた。スマトラの例ではヒト-ヒト伝播の統計学的エビデンスが見られ、概算された2次感染率は29%、局所的増殖数の下限値は1.14であった。トルコの例ではヒト-ヒト伝播のエビデンスは得られなかった。	
70162	2007/11/21	70750	鳥インフルエンザ	Lancet 2007; 370: 1137-1145	H5N1インフルエンザウイルスに感染した男性1名および妊婦1名とその胎児の剖検組織を調べた。肺のⅡ型上皮細胞、気管の上皮細胞、リンパ節のT細胞、脳の神経細胞及び胎盤のホフバウエル細胞と細胞栄養層でウイルス遺伝子配列と抗原が検出され、腸粘膜ではウイルス遺伝子配列のみが検出された。胎児では肺、末梢単核細胞、肝マクロファージに遺伝子配列と抗原が検出された。本ウイルスは肺だけでなく気管に感染し、脳を含む他の器官に拡がり、また胎盤を通過し、母親から胎児にも伝播しうる。	50
70103	2007/09/21	70533	鳥インフルエンザ	Transfusion 2007; 47: 452-459	血漿製剤の製造中に通常使われるウイルス不活性化処理、即ち、ヒトアルブミンのパストリ、静注用免疫グロブリン(IVIG)のSD処理、第Ⅷ因子インヒビターバイパス複合体製剤の蒸気加熱、及びIVIGの低pHインキュベーションが、H5N1インフルエンザウイルス不活化に有効かを再集合体株を使って調べた。その結果、H5N1インフルエンザウイルスは、エンベロープウイルスと同様の挙動を示し、これらのウイルス不活化処理によって効果的に不活化された。	
70103	2007/09/21	70533	伝染性紅斑	Vox Sanguinis 2007; 92: 121-124	ハプトグロビンおよび抗トロンビンの2つの異なった調整液にヒトパルボウイルスB19を加え、60℃で10時間処理した。異なった溶液中のB19は加熱中異なった熱感受性パターンを示し、ハプトグロビン調整液中では緩やかな不活性化、抗トロンビン調整液中では限定的な不活性化であった。異なった調整液を用いた以前の研究ではB19は迅速に不活性化され、今回の不活性化の動力学とは大きく異なった。B19の熱感受性は溶液組成に大きく依存する。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70165	2007/11/29	70768	日本脳炎	Epidemiol Infect 2007; 135: 974-977	2004年11月から2005年2月にかけて、日本の西部に位置する広島県の野生イノシシから血清25検体を採取した。日本脳炎ウイルス(JEV)に対する抗体検査を、IgMキャプチャー及びIgG酵素免疫測定法(ELISA)、並びにプラーク減少中和試験により行った。17検体(68%)がJEV中和抗体陽性だった。中和抗体陽性検体は全てIgG-ELISA陽性だった。1検体はIgMも陽性だった。約70%の野生イノシシが抗JEV抗体陽性であることが示され、この地域のJEV感染サイクルに関与している可能性が提示された。	51
70112	2007/09/28	70553	麻疹	asahi.com 2007年4月18日	東京都や埼玉県など関東地方ではしかが流行していることが、国立感染症研究所感染症情報センターがまとめた定点調査でわかった。例年より流行は早く、人の移動が活発になる連休に向けてさらに広がることが予想されるとして、同センターは緊急情報を出して注意を呼びかけている。同センターによると、例年、はしかの発症は乳幼児に多いが、今年の流行は10代前半や大人に多いのが特徴という。	

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 7. 18	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Mizoguchi H; Japanese Red Cross NAT Screening Research Group. Transfusion. 2007 Jul;47(7):1162-71.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○献血者のB型肝炎ウイルス血症及び抗原血症の期間:急性期におけるオカルト(B型肝炎表面抗原陰性)感染の予備的エビデンス

背景:日本赤十字社(JRC)は、血清学検査陰性献血者の検査のため、プールの全自動化及び核酸増幅検査(NAT)システムを導入した。JRCの保管検体と複数回献血に基づき、JRCスクリーニングシステムでB型肝炎表面抗原(HBsAg)及び抗B型肝炎コア抗原抗体が陰性であったB型肝炎ウイルス(HBV)DNA陽性献血者の遡及とフォローアップ検査を行った。

試験デザイン及び方法:2000年2月1日～2003年3月31日の期間に、半自動式多重検査システム(AMPLINAT MPX test, Roche)を用いて17,314,486本を50検体プールで検査した。当該期間に328本のHBV DNA陽性献血血液が見つかった。これらの献血者のうち26名から短期間に新たな検体を入手することができ、これにより急性HBV感染におけるウイルスマーカーの動態を調べることができた。ウイルス血症及び抗原血症の検出期間は、定量PCR法(JRC)とHBsAg酵素免疫測定法(Auszyme II, AxSYM, Abbott)、化学発光免疫測定法(Abbott)から得られた結果の回帰分析から推定した。

結果:個別NAT及び20検体ミニプール(MP) NATのHBV DNAの検出可能期間の中央値はそれぞれ74日及び50日と推定され、HBsAgの検出可能期間は42日と推定された。献血者26名中6名は変異型ウイルスに感染、この6名のうち3名はウイルス量が中等度(10^4～10^5 HBV DNA copies/mL)であったにもかかわらず、観察期間中に検出限界以上のHBsAgを発現しなかった。

結論:変異ウイルスの伝播は、急性期のオカルトHBV感染を引き起こすと考えられた。HBV NATは、MPで行ったとしても、HBsAg検査よりも効果的で、HBsAgウインドウ期前後の感染献血者を排除することができる。

報告企業の意見	今後の対応
献血時の核酸増幅検査で陽性となった献血者の検体を使用して急性HBV感染におけるウイルスマーカーの動態を調べたところ、個別NAT及び20検体ミニプール(MP) NATのHBV DNAの検出可能期間の中央値はそれぞれ74日及び50日、HBsAgの検出可能期間は42日と推定され、変異ウイルスの伝播は急性期のオカルトHBV感染を引き起こすと考えられたとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。NATの精度向上についても評価・検討している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen–negative) infection in the acute stage

Akira Yoshikawa, Yuko Gotanda, Kiyoshi Minegishi, Rikizo Taira, Satoru Hino, Kenji Tadokoro, Hitoshi Ohnuma, Keiko Miyakawa, Katsumi Tachibana, Hideaki Mizoguchi, and the Japanese Red Cross NAT Screening Research Group

BACKGROUND: The Japanese Red Cross (JRC) implemented a fully automated pooling and nucleic acid amplification test (NAT) system for testing seronegative donations. The JRC sample repository and repeat blood donations allowed for lookback and follow-up studies of hepatitis B virus (HBV) DNA–positive donors, who tested negative for hepatitis B surface antigen (HBsAg) and anti–hepatitis B core antigen in the JRC screening system.

STUDY DESIGN AND METHODS: From February 1, 2000, to March 31, 2003, 17,314,486 units were tested in 50-sample pools with a semiautomated multiplex assay system (AMPLINAT MPX test, Roche). During this period, 328 HBV DNA–positive donations were found. From 26 of these donors, sequential samples were available at short intervals. This enabled us to examine the dynamics of viral markers in acute HBV infection. The length of detectable periods of plasma viremia and antigenemia were estimated by regression analysis from the results obtained in the quantitative polymerase chain reaction assay (JRC) and HBsAg enzyme immunoassay (Auszyme II, AxSYM, Abbott) and chemiluminescence immunoassay (Abbott).

RESULTS: The median length of detectable HBV DNA in individual donation and 20-sample minipool (MP) NAT format was estimated to be 74 and 50 days, respectively, whereas the median length of detectable HBsAg was estimated to be 42 days. Six of the 26 donors were infected with mutant viruses, and 3 of these 6 donors did not develop detectable HBsAg during the entire observation period, despite a moderately high viral load of 10^4 to 10^5 HBV DNA copies per mL.

CONCLUSION: Transmission of mutant virus may cause occult HBV infection in the acute stage. HBV NAT, even in MP configuration, is more effective than HBsAg testing and capable of interdicting infected donors in the pre- and post-HBsAg window periods.

Studies on the early dynamics of hepatitis B viremia and antigenemia have placed an emphasis on comparing the sensitivity of hepatitis B virus (HBV) nucleic acid amplification test (NAT) methods and hepatitis surface antigen (HBsAg) assays in detecting early HBV infection with seroconversion panels.[1-4] We previously reported the dynamics of HBV DNA and HBsAg of genotypes A, B, and C in both the early increasing phase and the later decreasing phase of viremia with lookback and follow-up samples of 50 minipool (MP)-NAT–reactive donations.[5] With slight variation in the kinetics between the genotypes, a median HBV doubling time of 2.6 days was calculated in the ramp-up phase, which was the same as reported by Biswas and coworkers,[1] and the $t_{1/2}$ in the decreasing phase was 1.6 days. Regression analysis showed that the mean viral load at the HBsAg seroconversion point in chemiluminescence immunoassay (CLIA) was 2100 copies per mL.[5,6] A few HBsAg CLIA–negative donations, however, were found with relatively high viral roads between 10,000 and 100,000 copies per mL, which would be expected to be CLIA HBsAg–positive. To obtain a more accurate picture of the differences in

ABBREVIATIONS: CLIA = chemiluminescence immunoassay; HI = hemagglutination inhibition; ID = individual donation; JRC = Japanese Red Cross; MP = minipool; s/co = signal-to-cutoff.

From the Japanese Red Cross Saitama Blood Center, Hidaka, Saitama; and the Japanese Red Cross Headquarters, Blood Services Department, Minato-ku, Tokyo, Japan.

Address reprint requests to: Akira Yoshikawa, PhD, Japanese Red Cross Saitama Blood Center, 1370-12 Takahagi, Hidaka-shi, Saitama-ken 350-1213, Japan; e-mail: yoshikawa@saitama.bc.jrc.or.jp.

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early dynamics of HBV DNA and HBsAg relative to anti-hepatitis B core antigen (HBc) and alanine aminotransferase (ALT) response levels in the acute stage of HBV infection, we selected 26 blood donors of whom multiple sequential samples were available at relatively close intervals. This enabled us to compare the duration of detectable HBsAg with that of HBV DNA in both MP-NAT and individual donation (ID)-NAT format. Hereby not only the pre-HBsAg window period, but also the post-HBsAg window period was taken into account. This latter "core window period" is of less concern in Japan, because since 1989 all blood donations are tested for anti-HBc, but may be relevant for other highly endemic areas of hepatitis B in Asia, where anti-HBc testing is not performed. In this study special attention is given to a few donors with a relatively high viral load that remained HBsAg-negative during the entire follow-up period to anti-HBc seroconversion.

MATERIALS AND METHODS

MP-NAT

The NAT screening system in Japan was reported previously by Mine and colleagues.[7] In brief, all blood donations in the Japanese Red Cross (JRC) were from volunteers screened with a questionnaire survey administered by JRC blood centers throughout Japan. These donations were screened by serologic tests.[5-10] Serologically positive and elevated-ALT (>60 IU/mL) samples were excluded from NAT screening. NAT screening was carried out with a 50-sample pool with a multiplex (MPX) reagent (AMPLINAT MPX, Roche Diagnostic, Tokyo, Japan) specially ordered by JRC. The 95 percent HBV DNA detection limit of the AMPLINAT MPX test system was found to be 30 (range, 22-60) copies per mL based on a plasma standard quantified with an HBV RNA viral load assay (AMPLINAT MPX, Roche)[11] and was found to be 15 IU/mL (60 copies/mL) according to validation studies with the WHO standard by JRC.

Serologic screening tests

The JRC criterion for a serologically positive result is HBsAg positivity by reverse passive hemagglutination assay (sensitivity, 2 ng/mL) in the first screening and confirmed by enzyme immunoassay (EIA; AxSYM, Abbott Laboratories, Abbott Park, IL) or a high titer of the anti-HBc ($\geq 2^5$) without the antibody to HBsAg (anti-HBs; 200 mIU/mL). The titer of total (immunoglobulin G [IgG] + immunoglobulin M [IgM]) anti-HBc was determined by hemagglutination inhibition (HI) assay.[8] ALT was measured by machinery (ACA 5200, Olympus Co., Tokyo Japan) with reagents of transaminase HR-II (Wako Co., Tokyo, Japan).

Samples for studies

A total of 328 samples, collected from February 1, 2000, to March 31, 2003, were found positive by 50-pool NAT. Of these 328 donors, 26 had sampling intervals of less than 31 days and could be followed-up or looked back for both the increasing and the decreasing phase of viral load. Donors with sampling intervals longer than 32 days were excluded from analysis, because there might be a chance the viremia phase is missed by the sampling error (the shortest interval of HBV-DNA levels above 1200 copies/mL was 26 days) (Table 2, figures in bold).

Aliquots of all blood donations were stocked from September 1996 below –30°C for 10 years in two facilities in Japan.[7] When the donations were found to be HBV DNA–positive by pool-NAT (NAT-positive), the last stocked samples of the same donor were looked back by individual NAT. Of six donors, samples were obtained at short intervals with informed consent from hospitals, which enables us to study the course of serum markers in the recovery phase.

Serologic and individual NAT analyses of pool NAT-positive, follow-up, and lookback samples

Anti-HBc IgM was detected with EIA (IMxHBc-M, Abbott Laboratories). HBsAg detection by EIA was carried out with HBsAg testing by overnight method (Auszyme II, Abbott Laboratories, until October 2002; and AxSYM HBsAg, Abbott Laboratories, was adopted thereafter). Some samples were retested by CLIA (PRISM and Architect, Abbott Laboratories). Anti-HBs levels were measured by passive hemagglutination assay (JRC in-house reagent) and EIA (AxSYM, Abbott Laboratories). Data were arranged according to the manufacturer's instructions. The qualitative and quantitative detections of HBV DNA by individual NAT were reported previously by Minegishi and coworkers.[6]

The genotypes of HBV and precore or core promoter mutations were determined and characterized according to Okamoto and coworkers.[12,13] The lengths of viremia (HBV DNA load greater than 60 or 1200 copies/mL) and antigenemia were measured directly from log-linear lines of each of the 26 figures, because the slope of ramp-up curve was peculiar to each sample as shown in Figs. 1 through 3.

Statistical analysis

For estimation of the length of HBV DNA MP-NAT– and ID-NAT–detectable periods, we had to make a number of assumptions: 1) The increase and decrease in HBV DNA viral load and HBsAg signal-to-cutoff (s/co) follows a log-linear relationship with time.[5] 2) Taking into account the present uncertainty in standardizing HBV DNA assays in

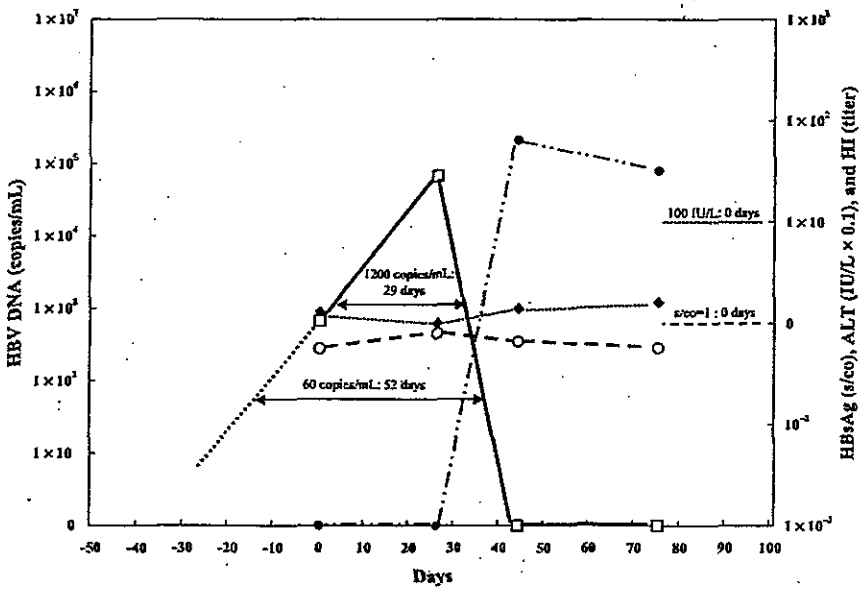

Fig. 1. Course of occult HBV infection. The viremia period with HBV DNA loads greater than 1200 copies per mL is 29 days. HBV DNA is first detected by MP-NAT on Day 0. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day 0 (straight line). Undetectable HBV DNA load was assumed 1 copy per mL (intrapolation). The viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 52 days (shown in italics). The s/co (S/N/2) value of HBsAg EIA (○; AxSYM, Abbott Laboratories) level remained below one during observation. The ALT (◆) level also remained less than 100 IU per L during observation even after anti-HBc titer (HI, ●) increased. The maximum HI titer is 2^6. The donor whose results are shown in Fig. 1 corresponds to Donor 3 in Tables 1-3.

copies per mL, we assumed a 95 percent detection limit of the AMPLINAT system in ID-NAT of approximately 60 copies per mL. For the current 20-donation MP-NAT system in use, we therefore assume a cutoff HBV DNA level of 1200 copies per mL above which HBV DNA is detected with greater than 95 percent certainty. 3) AMPLINAT-reactive donations below the quantification limit in the JRC quantitative polymerase chain reaction assay were assumed to have a viral load of 60 copies per mL. In fact, these samples could also have had a lower viral load around the 50 percent detection limit and therefore the observed periods might be longer when estimated at the approximately 60 copies per mL cutoff level (Table 2, figures in lightface text). For the HBV DNA–detectable periods that were determined by extrapolation (Table 2, figures in italics), however, the estimates of HBV DNA–detectable periods may have been shorter, because intervals could be two to three doubling times (5-8 days) or half-times (3-5 days) longer at the 50 percent AMPLINAT detection limit. 4) An AMPLINAT-negative sample in the previremic or the early recovery phase that was required for estimating the slope of the decreasing viral load was assumed to have a concentration of 1 copy per mL. For estimation of the length of periods of detectable HBV DNA above 1200 and 60 copies per mL, this is a conservative estimate, because AMPLINAT may have failed to detect higher HBV DNA levels in the range between the 50 to 95 percent detection limit (estimated, 10-60 copies/mL). If this was the case, HBV DNA levels would increase or decrease slower and the HBV DNA–detectable period above 60 copies per mL would be estimated to be shorter. The U test and Yates chi-square test were used for statistical analysis.

RESULTS

Of 328 NAT-positive donations, 26 were from donors who could be followed-up in both increasing and decreasing phases of HBV DNA load. The data of the 26 donors including genotype, subtype, mutation, sex, age, sample numbers, and period from ID-NAT–positive (>60 copies/mL) to anti-HBs seroconversion are shown in Table 1. The lengths of the viremia and antigenemia, peak HBV loads, ALT levels, HI titers (total IgG + IgM anti-HBc), and periods from pool NAT-positive (>1200 copies/mL) day to the HBV DNA load peak and the ALT level or HI titer peak are shown in Table 2. Data shown in Tables 1 and 2 are arranged according to the lengths of the HBsAg EIA–positive period. There are data sets of different quality in Table 2 (data in bold, lightface, and italics) as shown in the footnote of Table 2. For statistical comparison between HBV DNA– and HBsAg-detectable periods we take those donors into account that have bold figures for both HBsAg and HBV-DNA (Donors 5, 10, 15, 19, 21, and 24). The length of viremia (>1200 copies/mL; MP-NAT) was longer than that of antigenemia in Donors 5, 10, and 15 and length of antigenemia was longer than that of viremia in Donors 19, 21, and 24. Peak HBV DNA loads of Donors 19, 21, and 24 were higher than those of Donors 5, 10, and 15 (U test, $p < 0.05$). Median length of viremia (>1200 copies/mL) was 62 days (range, 26-99 days) and that of antigenemia was 56 days (range, 23-129 days). When we take into account donors with bold and regular printed data ($n = 15$; intrapolation of assumption, <100 copies/mL: 60 copies/mL, undetectable: 1 copy/mL), 10 donors had longer viremia (>1200 copies/mL) than antigenemia periods, one had same period and four had longer antigenemia than viremia (>1200 copies/mL). Median length of viremia (>1200 copies/mL) was 58 days (range,

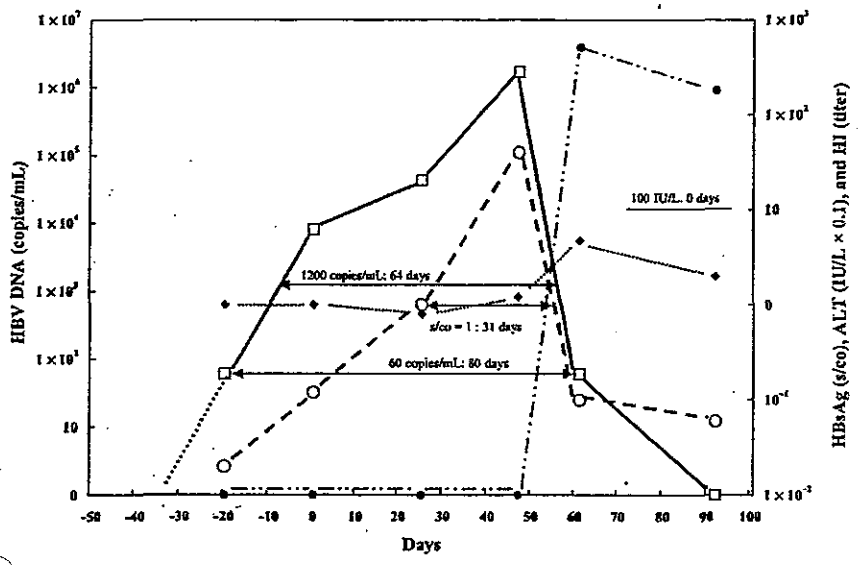

Fig. 2. Course of a case that ALT levels remained less than 100 IU per L. The viremia period with HBV DNA loads greater than 1200 copies per mL is 64 days. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day –20 (straight line). HBV DNA load below 100 copies per mL was assumed 60 copies per mL. The viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 80 days. The HBsAg EIA (○, Auszyme II, Abbott Laboratories)-positive period is 31 days. The s/co datum of Day 47 is greater than 40. Maximum HI titer is 2^9. The donor whose results are shown in Fig. 2 corresponds to Donor 10 in Tables 1 through 3. (◆) ALT; (●) HI.

26-108 days) and that of antigenemia was 48 days (range, 23-129 days). When we compare the detectable length between viremia and antigenemia of all data (n = 26) including italic (slopes are based on assumption of timing of viremia peak and estimates are obtained by extrapolation), median length of viremia (>1200 copies/mL) was 50 days (range, 23-116 days), and that of antigenemia was 12 days (range, 0-175 days; U test, not significant). Eight donors had longer HBsAg-detectable period than viremia period above 1200 copies per mL. In contrast, median length of viremia above 60 copies per mL (ID-NAT; most of them were italic because of the estimation by extrapolation) was 74 days (range, 43-213 days) and all donors had longer viremia period than HBsAg-detectable period (U test, $p < 0.01$).

HBsAg was not detected during observation in three donors: "occult" HBV infection (Donors 1-3 in Tables 1-3). One of such donors (Donor 3) is shown in Fig. 1. The three donors showed peak HBV DNA loads of less than 10^5 copies per mL and peak ALT levels less than 100 IU per L. The data of these three cases are as follows: genotype C, subtype adr, and precore or core promoter mutants (Tables 1-3 and Fig. 4). The maximum viral loads of Donors 4, 5, and 8 were less than 10^5 copies per mL (8,200-80,000 copies/mL) but periods of detectable HBsAg were 11 to 28 days, although the maximum viral loads of Donors 1 through 3 were 20,000 to 68,000 copies per mL and HBsAg was undetectable (Tables 2 and 3). To examine the difference, we compared the DNA sequences of core promoter and precore region (nucleotides 1699-1780 and 1885-1919) and surface region (nucleotides 535-594) between Donors 1 to 5 and 8 (Fig. 4). The mutations in core-promoter region (nucleotides 1762 and 1764; Donor 2) and precore region (pC-28; Donors 1 and 3) were observed in "occult" cases. No difference was observed in sites of escape mutations except for S-129 (Q129N) of Donor 1. Because Donor 1 had no clear evidence of anti-HBc (HI:2^5, anti-HBc[IgM]:negative) and anti-HBs seroconversion, we could not exclude the possibility that Donor 1 was a late-stage typical occult carrier. Donors 2 and 3, however, showed clear seroconversion to anti-HBc(IgM) and anti-HBs and could be classified as "occult HBV infections in acute stage" because the viral loads reached levels of 29,000 and 68,000 copies/mL, whereas HBsAg response remained at or below cutoff level (Table 3).

Although we had already reported that the precore and core promoter mutations affect the doubling time of HBV DNA loads,[5] the effect of the mutations on HBsAg production is unclear. When comparing peak viral loads and HBsAg detection in mutant and wild-type infections, there were significant differences between them (peak HBV DNA loads, $p < 0.05$; length of HBsAg detection periods, $p < 0.01$; U test). The nucleic acids from nucleotide 1374 to nucleotide 1838 encode X protein. Mutations at nucleotide 1719 and nucleotide 1721 change the amino acids from leucine to valine (x-116), nucleotide 1762 change the amino acid from lysine to methionine (x-130), and nucleotide 1764 change the amino acid from valine to isoleucine (x-131). Though the function of X protein has not been established, mutations of X-protein might affect the production of HBsAg.[14] Mutations in core promoter or precore, however, were found in Donors 7, 11, and 13 whose HBsAg detectable periods were 28 to 40 days. We should compare the sequence of the other regions between Donors 1 to 5, 7, 8, 11, and 13.

The peak ALT level was maintained below 100 IU per L in 12 donors (Table 2). One of these 12 cases is shown in Fig. 2 (Donor 10). About 5 donors with the shortest intervals (Donors 19, 21, 22, 24, and 26; Table 2), course of markers (ALT/anti-HBc vs. HBV-DNA timing analysis) were compared. The peak viral load preceded the peak

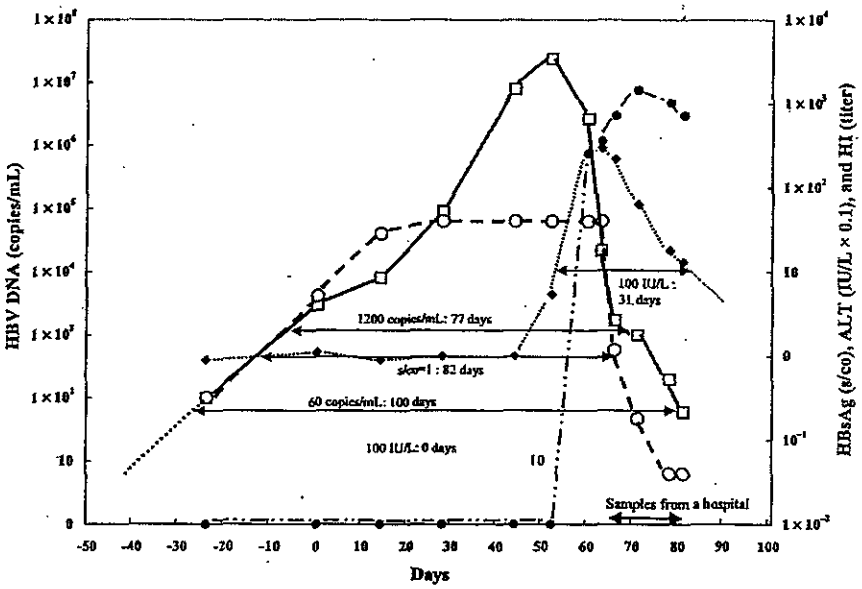

Fig. 3. Course of a symptomatic HBV infection. The viremia period with HBV DNA loads greater than 1200 copies per mL is 77 days. The estimated line is drawn (dotted line, extrapolation) from the log-linear line on Day –24 (straight line). HBV DNA load below 100 copies per mL was assumed to be 60 copies per mL. The length of viremia period with HBV DNA (□) loads greater than 60 copies per mL is estimated to be 110 days (shown in italics). The length of the HBsAg EIA (○; Auszyme II, Abbott Laboratories)-positive period is 82 days, longer than the viremia period with HBV DNA loads greater than 1200 copies per mL (77 days). The s/co data on Days 29, 45, 53, 61, and 64 are greater than 40. The ALT (◆) level on Day 63 is 2973 IU per L. The estimated line of ALT is drawn (thick dotted line) from the log-linear line (thin dotted line) on Day 81. The symptomatic period with ALT levels more than 100 IU per L is estimated to be 31 days (shown in italics). Maximum HI (●) titer is $2^{10.5}$. After Day 63, samples were obtained from a hospital with informed consent. The donor whose results are shown in Fig. 3 corresponds to Donor 21 in Tables 1 through 3.

ALT and anti-HBc level with approximately 2 weeks (ALT, 14, 11, 12, 23, and 13 days; anti-HBc, 14, 19, 12, 16, and 18 days; Table 2, bold). The case of Donor 21 is shown in Fig. 3. The lengths from individual NAT-positive (>60 copies/mL) day to anti-HBs seroconversion day (>10 mIU/mL) could be measured in 17 donors, ranges from 33 to 188 days (Table 1).

DISCUSSION

The JRC has established a sample repository of all donations, which enables doing valuable lookback studies on donors that were found AMPLINAT reactive in the JRC MP screening system. In a previous report, we have already described the dynamics of viremia in donors that were identified by the JRC MP-AMPLINAT screening system and of whom follow-up samples were available in either the increasing phase or the decreasing phase of viremia.[5] The data, however, did not allow for accurate timing of the peak of viremia in most of the donors and therefore the total length of the AMPLINAT-detectable period could not be reliably established. From a blood safety perspective comparison of the total lengths of the periods of detectable HBsAg and HBV DNA in either MP- or ID-NAT format is of interest. To be able to estimate these periods we selected 26 donors of whom samples were available in both the increasing and decreasing phase of viremia and whose sampling intervals were less than 1 month (the shortest time of detectable viremia found in our donor follow-up studies). The follow-up data were used to estimate the time periods where the viral load was higher than 1200 copies per mL (the estimated 95% detection limit of the current 20-donation MP-AMPLINAT system of JRC) and above 60 copies per mL (the estimated 95% detection limit of AMPLINAT in ID-NAT format). A similar calculation method was followed to estimate HBsAg-detectable periods based on the assumption that HBsAg s/co values follow a similar log-linear relationship with time in the ramp-up phase and recovery phase as assumed for HBV DNA donors (which assumption may be not totally correct). When we based our calculations on the most reliable data of the subset of six donors, the median MP-NAT–detectable period (>1200 copies/mL) was 62 (range, 26-109) days, whereas the ID-NAT–detectable period (>60 copies/mL; not so reliable data as ID-NAT) was 84 (range, 45-155) days. These periods were longer than the median HBsAg-detectable periods, which in the same donors was found to be 56 (range, 23-129) days. A similar result was found when all data were taken into account including the calculated periods based on the subset of donors in whom the dynamics of viremia were based on assumptions of viral load in some of the samples (see Results, Table 2).

Interestingly, we found 3 of the 26 donors (11.5%) who during the entire course of acute infection never developed HBsAg levels above the cut of CLIA and EIA, whereas HBV-DNA levels in these 3 donors reached levels as high as 20,000, 29,000, and 68,000 copies per mL. We are tempted to classify these cases as occult HBV infection in the acute phase, because normally the HBsAg CLIA assay becomes detectable in acute infection when HBV DNA reaches a concentration above approximately 2,000 copies per mL.[1,3,6,4] So far, occult HBV infection has

TABLE 1. Data of 26 HBV NAT–positive donors

Donor	Genotype	Subtype	Wild/ mutant*	Age (years)	Sex	Number of serial samples		Observation periods§ (days)	Sero-conversion‖ (days)
						Lookback†	Follow-up‡		
1	C	adr	M(C)¶	55	Male	0	5	105	—
2	C	adr	M (pro)	20	Female	1	3	92	62
3	C	adr	M (C)	48	Female	0	4	75	75
4	C	adr	W	24	Female	0	5	59	59
5	C	adr	W	23	Female	0	4	59	59
6	C	adw	W	21	Female	0	4	55	55
7	C	adr	M (C)	19	Female	0	3	33	—
8	C	adr	W	24	Female	1	4	99	33
9	C	adr	W	25	Male	2	3	91	75
10	C	adr	W	24	Female	1	5	112	112
11	C	adr	M (C&pro)	24	Female	0	3	44	85**
12	C	adr	W	26	Female	1	3	75	75
13	C	adw	M (pro)	36	Female	0	4	43	43
14	A	adw	W	31	Male	0	3	56	56
15	C	adr	W	24	Male	0	4	56	—
16	C	adr	W	18	Female	0	4	52	—
17	C	adr	W	26	Male	0	4	74	74
18	C	adr	W	29	Male	0	5	105	—
19	C	adr	W	25	Female	0	13	231	188
20	C	adr	W	47	Male	0	5	103	—
21	C	adr	W	23	Female	1	11	105	145**
22	B	adw	W	23	Female	0	21	134	186**
23	C	adr	W	22	Male	0	7	145	—
24	C	adr	W	27	Male	2	14	149	177**
25	B	adw	W	30	Male	0	5	105	—
26	C	adr	W	31	Male	0	20	224	—

* (C) = precore mutation; (pro) = core-promoter mutation.
† Number of looked-back (stored) samples.
‡ Number of followed-up samples including 50 pool NAT-positive sample.
§ Intervals of blood collection are less than 31 days. Looked-back (individual NAT negative) days were included.
‖ Length from individual NAT-positive day to HBsAb seroconversion (more than 10 mIU/mL) days. HBsAb levels (10 mIU/mL) were determined by EIA (AxSYM, Abbott Laboratories). — = HBsAb (more than 10 mIU/mL) was not detected during observation except for Donor 1. Anti-HBs of Donor 1 could not be tested by AxSYM (Abbott) as shown in Table 3.
¶ In addition to precore mutation, a mutation of surface region (Q129N) was observed as shown in Fig. 4.
** Some donors come to check their HBsAb seroconversion and disappearance of HBsAg after a long interval.

been defined as HBsAg-negative, but persistent HBV DNA–positive infection in a late stage, either tail end chronic carriers who over a prolonged period of time no longer produce detectable HBsAg or recovered individuals with recurrent virus replication after anti-HBs levels, have dropped to low titers.[15,16,17] The HBV DNA–positive, but HBsAg-negative infections found in the pre- and post-HBsAg window phases during acute infection (as shown in this study) should of course not be confused with the definition of occult infection. This is the normal pattern of acute infection that we have observed in 20 donors infected with wild-type virus in this study (Tables 1 and 2). In 6 donors, however, we have been able to identify core promoter or precore mutations that typically develop over time in chronic infection and are associated with seroconversion from HBeAg to anti-HBe and with lower HBV DNA and HBsAg levels.[18] We already reported that precore mutations were associated with longer viral doubling times in our acutely infected donors.[5] Most likely, these mutations already had developed in chronic carriers of the HBV genotype C strains that were transmitted to these donors. In the present study, 3 of 6 donors infected with mutant virus did not produce detectable HBsAg, whereas all 20 donors infected with wild-type virus did (Yates chi-square test, $p < 0.01$).

Therefore, the presence of core promotor and precore mutations in the transmitted virus may have effects on the HBV DNA and HBsAg expression in the recipient and be associated with a higher risk of an occult (HBsAg-negative) course or fulminant hepatitis[19-21] in the acute stage. Infection with these mutations, however, is not predictive for an occult course in the acute phase, because we saw three donors infected with mutant virus (Donors 7, 11, and 13) that developed detectable HBsAg for an estimated period of 28 to 40 days. So it may be that also host factors play a role or it may be that mutations in other regions of the genome (e.g., pre-S region) account for defect in HBsAg expression. To examine this we have to sequence other regions of the genome.

In one of the three donors with occult HBV infection (Donor 1), we also were able to identify a mutation in the S region of the genome (Q129N) at an amino acid

TABLE 2. Analysis of 26 HBV NAT–positive donations from donors who could be followed-up from early stage of infection to end of viremia

	Length of period (days)*				Peak or maximum value and the time (days from pool NAT-positive [>1200 copies/mL])†					
	HBV DNA (copies/mL)			ALT (IU/L)	HBV DNA		ALT		Anti-HBc	
No Donor	>1200	>60	HBsAg-positive	>100	Copies/mL	Days	IU/L	Days	HI: 2ⁿ	Days
1	*49*	*90*	*0‡*	0	2.0×10^4	*40*	48	—	>5§	*69*
2	*23*	*43*	*0*	0	2.9×10^4	*14*	14	—	10	*45*
3	*29*	*52*	*0*	0	6.8×10^4	*23*	16	—	6	*41*
4	*25*	*59*	*11*	0	8.2×10^3	*19*	10	—	>8	*>61*
5	**26**	*45*	**23**	0	8.0×10^4	*17*	36	—	>8	*>61*
6	*36*	*59*	*24*	0	1.2×10^5	*29*	19	—	>6.5	*>74*
7	*36*	*58*	*28*	0	1.4×10^5	*25*	10	—	>7.5	*>46*
8	33	53	28	26	5.5×10^4	*23*	323	*44*	10	*44*
9	29	46	29	0	1.4×10^5	*17*	22	—	7	*45*
10	**64**	80	**31**	0	1.7×10^6	*54*	47	—	9	*68*
11	*50*	*69*	*34*	0	5.6×10^6	*29*	23	—	11	*58*
12	56	75	34	0	9.8×10^5	*36*	>71	—	>8.5	*>64*
13	*39*	*56*	*40*	>4	7.5×10^5	*22*	139	>45	>8	*>45*
14	49	*62*	*44*	>7	4.6×10^6	*34*	>257	>54	>8	*54*
15	**49**	*73*	**45**	6	9.8×10^4	*30*	142	*44*	>7	*>58*
16	*42*	*66*	*46*	23	1.5×10^6	*22*	1705	*38*	>10	*38*
17	52	*83*	51	0	1.4×10^6	*37*	50	—	>9	*>68*
18‖	*75*	*112*	*61*	48	2.5×10^8	*49*	265	*49*	11	*78*
19	**60**	*87*	**68**	17	9.6×10^6	35	893	**49**	11	**49**
20‖	73	*116*	69	33	1.2×10^7	*28*	1395	*58*	11	*58*
21‖	*77*	*110*	**82**	31	2.4×10^7	**60**	2973	**71**	10.5	**79**
22‖	93	*121*	105	50	4.8×10^8	**60**	1467	**72**	11	**72**
23	108	*170*	98	>8	8.4×10^6	*85*	>309	*>107*	>10	*>107*
24‖	**99**	155	**129**	64	2.8×10^7	*77*	1707	**100**	11.5	**93**
25	*98*	*155*	*104*	43	6.8×10^8	*55*	408	*87*	11.5	*87*
26‖	*113*	*213*	*175*	38	1.1×10^9	**58**	2792	**71**	8	**76**

* Data in bold when slopes of curves in increasing and decreasing phase are based on actual viral load or s/co measurements with an observed HBV DNA or HBsAg peak (the intervals of sequential samples of around peak were within 15 days) and estimates are obtained by intrapolation and not by extrapolation to lower assumed ID-NAT concentration. Data in lightface when slopes of curves in increasing and decreasing phase are based on assumption of timing of viremia peak and estimates are obtained by intrapolation with assumption of the viral load such as approximately 60 copies per mL or approximately 1 copy per mL in quantitative PCR-negative donations with and without AMPLINAT reactivity. Data in italics when slopes are based on assumption of timing of viremia peak and estimates are obtained by extrapolation with assumption of the viral load such as approximately 60 copies per mL or approximately 1 copy per mL.

† Data in bold show that intervals of sequential samples around the HBV DNA or HBsAg peak are within 15 days (most of samples were within an interval of 7 days). Data in italics show that MP-positive date was estimated by intrapolation or extrapolation.

‡ Zero means occult HBV infection (HBsAg EIA is negative during observation).

§ > = the data are not the peak but maximum values during observation. Because of the incomplete follow-up, no peaks could be obtained.

‖ — = ALT was less than 100 IU per L during observation. Some samples are obtained from hospitals with informed consent.

position known to be associated with occult HBV infection.[22] In fact we are not certain that this donor has been infected with mutant virus and developed acute HBV infection, because the only indication is a borderline detectable titer in the JRC HI screening method and a drop in HBV DNA titer. Further follow-up testing is required to confirm delayed anti-HBc and anti-HBs conversion in this particular case. The laboratory results may also represent chronic occult HBV infection without detectable anti-HBc and fluctuating viral load. Whatever is the natural course, detection of HBV DNA was the only tool to identify occult infection in this donor. Our study shows that NAT is not only valuable for detection of low HBV DNA levels in the pre- and post-HBsAg window periods, but also may be capable of identifying higher levels of viremia in anti-HBc–negative donors with occult HBV infection in either an acute or chronic stage. The reduction in pool size from 50 to 20 donations in our MP NAT screening system from August 2004, may be instrumental in interdicting such donations with moderate levels of HBV DNA and so further reduce the risk of post-transfusion HBV infection in Japan.

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TABLE 3. Change of HBV-related markers of serial samples from pool NAT-positive donors who could be looked-back and followed-up

No Donor	Time (days) from MP-NAT–positive day*	ALT Transaminase HR-II (IU/L)	HBV DNA ID-NAT TaqMan (copies/mL)	HBsAg EIA Auszyme II (s/co)†	HBsAg EIA AxSYM (s/co)†	HBsAg CLIA Architect (IU/mL)‡	HBsAg CLIA PRISM (s/co)†	HBcAb EIA HI (2^n)	HBcAb EIA AxSYM (% Inh)§	HBcAb EIA IMx HBc-M indexII	HBcAb EIA PHA (2^n)¶	HBcAb EIA AxSYM (mIU/mL)
1	0	48	2.3×10^2	0.06	NT**	0.01	0.50	0	9.30	0.04	0	NT
	21	45	3.1×10^2	0.10	NT	NT	NT	0	NT	0.04	0	NT
	43	33	1.9×10^3	0.04	NT	NT	NT	0	NT	0.04	0	NT
	74	41	2.0×10^4	0.08	NT	0.01	NT	0	24.32	0.04	0	NT
	105	36	—	0.04	NT	NT	NT	5	NT	0.05	0	NT
2	–30	10	—	0.18	NT	NT	NT	0	2.40	0.06	0	NT
	0	10	2.9×10^4	0.46	NT	NT	0.99	0	NT	0.07	0	NT
	31	14	—	0.1	NT	NT	NT	10	87.70	2.55	0.5	0.1
	62	8	—	0.14	NT	NT	NT	8.5	93.00	2.02	4.5	199.2
3 (Fig. 1)	0	13	6.8×10^2	NT	0.56	0.02	0.37	0	NT	0.07	NT	0
	26	10	6.8×10^4	NT	0.79	0.04	NT	0	22.05	0.07	0	0
	44	14	—	NT	0.61	0.03	NT	6	65.55	1.49	0	0
	75	16	—	NT	0.57	0.00	NT	5	75.51	1.11	4	97.9
10 (Fig. 2)	–20	10	$<1.0 \times 10^2$	0.02	0.75	NT	NT	0	NT	0.04	0	0
	0	10	8.1×10^3	0.12	NT	NT	0.40	0	NT	0.05	0	NT
	25	8	4.3×10^4	1.00	1.23	NT	NT	0	NT	0.05	0	0
	47	12	1.7×10^6	>40	65.69	NT	NT	0	NT	0.05	0	0
	61	47	$<1.0 \times 10^2$	0.1	0.66	NT	NT	9	NT	2.06	0	0.9
	92	20	—	0.06	0.58	NT	NT	7.5	NT	1.31	1	36.5
21 (Fig. 3)	–24	9	1.0×102	0.32	0.38	NT	NT	0	NT	0.06	0	0
	0	11	3.0×10^3	5.24	NT	NT	6.92	0	NT	0.07	0	NT
	14	9	8.1×10^3	28.62	6.68	NT	NT	0	NT	0.07	0	0
	28	10	9.1×10^4	>40	26.83	NT	NT	0	NT	0.06	0	0
	44	10	7.9×10^6	>40	53.00	NT	NT	0	NT	0.06	0	0
	52	54	2.4×10^7	>40	80.37	NT	NT	0	NT	0.06	0	0
	60	2567	2.7×10^6	>40	73.29	NT	NT	8	NT	1.66	0	0
	63	2973	2.2×10^4	>40	12.1	NT	NT	8.5	NT	2.2	0	0.8
	66	2199	1.7×10^3	1.18	0.61	NT	NT	9.5	NT	2.36	0	1.4
	71	635	1.0×10^3	0.18	0.33	NT	NT	10.5	NT	2.44	0	1.7
	78	179	2.0×10^2	0.04	0.40	NT	NT	10	NT	2.50	0	1.3
	81	131	$<1.0 \times 10^2$	0.04	0.39	NT	NT	9.5	NT	2.44	0	1.0
	121††	20	—	0.02	0.45	NT	NT	8.5	NT	1.89	0	10.4

* Day (0) = 50-pool NAT-positive day; – = looked-back by stored samples.
† s/co: ≥1 = positive; <1 = negative.
‡ IU/mL: ≥0.05 = positive; <= 0.05: negative.
§ % inhibition: ≥50% = positive; <50% = negative.
‖ Index: ≥1.2 = positive; 0.8-1.2 = indeterminate; <0.8 = negative.
¶ 2^4 corresponds to 200 mIU/ml.
†† The interval was more than 32 days.
NT = not tested.

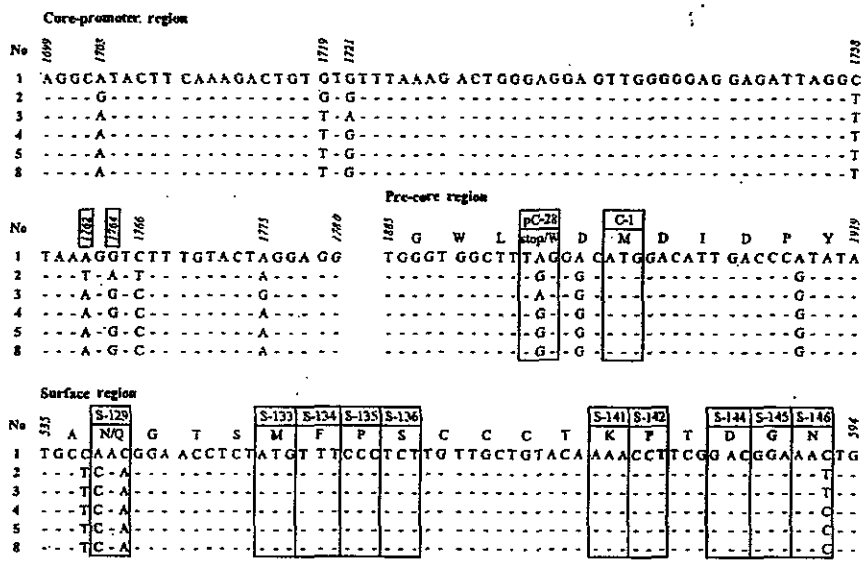

Fig. 4. The sequence of the HBV core-promoter (nucleotides 1699-1780), precore (nucleotides 1885-1919), and surface (nucleotides 535-594) region of Donors 1 to 5 and 8. The characters of HBV of all donors were genotype C and subtype adr. Donor 2 has core-promoter mutations (nucleotides 1762:A/T and 1764:G/A; nucleotides number are boxed). Donors 1 and 3 have precore mutations (nucleotide 1896; precore codon 28). The amino acids of precore and core are shown at the top of the sequences and precore codon 28 and first core codon: methionine are boxed. Donor 1 has an escape mutation (nucleotides 539:C/A and 541:A/C, codon 129:Q/N). The amino acids reported as escape mutations[5] are boxed and the number is shown at the top of the box. Nucleotide was numbered according to Okamoto and coworkers.[13]

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研究報告の概要

○遡及調査で特定されたB型肝炎ウイルスDNA値の低い血液製剤の感染性

背景:日本赤十字社(JRC)は、HBc抗体スクリーニングを1989年に、50検体ミニプール(MP)核酸増幅検査を2000年に導入した。B型肝炎表面抗原(HBsAg)発現前やMP-NATウインドウ期に採血された献血血液並びにオカルトHBV感染献血者由来の献血血液のB型肝炎ウイルス(HBV)伝播リスクを調べるために、系統的遡及調査が実施されている。

試験デザイン及び方法:JRCは、1996年以降、各献血血液の一部を保管している。この完全な保管検体システムに基づき、1997年～2004年の期間に得られた複数回献血者の全献血血液の遡及調査を行った。複数回献血者がHBVウイルスマーカー陽性となったときには、当該献血者の過去の献血血液の保管検体を個別(ID)-NATにより検査した。ID-NATのみ陽性である献血血液の頻度と、そのような血液由来の血液製剤の輸血によるHBV伝播リスクを検討した。

結果:15,721本の保管検体にHBV ID-NATを実施したところ、158検体 (1.01%) がHBV DNA陽性となった。この158検体のうち、95 (60%) はHBc抗体価の低い献血者由来であった。ID-NATのみ陽性の献血血液由来製剤を輸血された63名のうち、HBV感染が確認されたのは12名(19%)であった。HBc抗体価の低い血液製剤33製剤のうち感染性が特定されたのは1製剤のみであったが、HBc抗体陰性製剤では、22製剤中11製剤に感染性が認められた。HBs抗体陽性であると特定された16製剤のうち、血清学検査により感染の証拠を示した製剤はなかった。

結論:JRCのスクリーニングシステムをすり抜けたHBc 抗体価の低いオカルトHBV感染者由来の血液製剤を原因とする臨床的に観察されたHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期に献血された血液の伝播リスクよりも10倍以上低い。

報告企業の意見	今後の対応
日本赤十字社のスクリーニングシステムをすり抜けたHBc抗体価の低いオカルトHBV感染者由来の血液製剤を原因とするHBV感染リスクは、HBsAg発現前やMP-NATウインドウ期に献血された血液の伝播リスクよりも10倍以上低いとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。NATの精度向上についても評価・検討している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

(2)

Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program

Masahiro Satake, Rikizo Taira, Hisao Yugi, Satoru Hino, Kimihiro Kanemitsu, Hisami Ikeda, and Kenji Tadokoro

BACKGROUND: Japanese Red Cross (JRC) blood centers implemented anti-hepatitis B core antigen (HBc) screening in 1989 and 50-minipool (MP)-nucleic acid testing (NAT) in 2000. A systematic lookback study has been conducted to determine the hepatitis B virus (HBV) transmission risk of donations drawn in the pre-hepatitis B surface antigen (HBsAg) and/or MP-NAT window phase and by donors with occult HBV infection.

STUDY DESIGN AND METHODS: JRC blood centers have been storing aliquots of every blood donation since 1996. On the basis of the complete repository tube archives, all donations from repeat donors received from 1997 to 2004 were subjected to a lookback study. When repeat donors turned positive for HBV viral marker(s), repository tubes from their previous donations were tested for HBV with individual-donation (ID)-NAT. The frequency of ID-NAT-only–positive donations and the HBV transmission risk by the transfusion of those components were investigated.

RESULTS: HBV ID-NAT was performed on 15,721 repository tubes, and 158 tubes (1.01%) were found positive for the presence of HBV DNA. Of these 158 ID-NAT-only–positive donations, 95 (60%) were derived from carriers with low anti-HBc titers. Of 63 patients transfused with ID-NAT-only–positive components, 12 (19%) proved to be infected with HBV. Only 1 of 33 components with low anti-HBc titers could be identified as infectious, whereas 11 of 22 anti-HBc–negative components proved to be infectious. None of the 16 identified hepatitis B surface antibody–positive components showed serologic evidence of infection.

CONCLUSION: The clinically observed HBV infection risk caused by blood components from occult HBV carriers with low anti-HBc titers who slip through the JRC screening system is more than 10-fold lower than the transmission risk by donations in the pre-HBsAg and/or MP-NAT window phase.

Nucleic acid testing (NAT) for hepatitis C virus (HCV) and human immunodeficiency virus (HIV) has been implemented in developed countries for screening blood donated during the window period and plays a critical role in excluding infectious blood components.[1,2] For hepatitis B virus (HBV), Japanese Red Cross (JRC) blood centers have been screening blood with hemagglutination for hepatitis B surface antigen (HBsAg) detection combined with anti-hepatitis B core antigen (anti-HBc) and hepatitis B surface antibody (anti-HBs) testing since 1989 and implemented 50-member-pool NAT (50-NAT) for HBV, HCV, and HIV in February 2000.[3,4] There is still a residual risk of transfusion-transmitted viral infection (TTI), however, because of the limited sensitivity and use of pooled samples in current NAT systems.[5] A precise evaluation of residual risk after NAT implementation is essential in determining whether further strategies for preventing TTI are warranted (e.g., individual donation [ID]-NAT or pathogen reduction).[6,7]

To investigate the cause of reported TTI and to identify and retrieve virus-containing components, JRC has been storing aliquots of every blood donation since 1996 with a plan to preserve repository aliquots for 11 years. A

ABBREVIATIONS: 50-NAT = 50-member-pool nucleic acid testing; HBs = hepatitis B surface antibody; HI = hemagglutination inhibition; ID = individual donation; JRC = Japanese Red Cross; MP = minipool; TTI = transfusion-transmitted viral infection.

From the Tokyo Red Cross Blood Center, Tokyo; the Hokkaido Red Cross Blood Center, Sapporo; and the Blood Service Headquarters, Japanese Red Cross Society, Tokyo, Japan.

Address reprint requests to: Masahiro Satake, Tokyo Red Cross Blood Center, 2-1-67, Tatsumi, Koto-ku, Tokyo, Japan, 135-8639; e-mail: ma-satake@tokyo.bc.jrc.or.jp.

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systematic lookback study has been conducted in which repository tubes from previous donations were investigated for the presence of HBV, HCV, or HIV sequences by ID-NAT when repeat donors turned positive for viral antigens, anti-viral antibodies, or screening 50-NAT.[8] In the present lookback study, the proportion of ID-NAT–reactive donations that are not detected by the current JRC blood screening algorithm has been established and the clinically observed HBV transmission risk of the components derived from these donations has been investigated.

MATERIALS AND METHODS

In JRC blood centers, all donated blood samples are first screened for HBsAg by reversed passive hemagglutination,[9] the sensitivity of which is 3 ng per mL. HBsAg-negative samples are further screened for anti-HBc and anti-HBs by hemagglutination inhibition (HI) and particle agglutination, respectively. Only blood having a high anti-HBs titer ($\geq 2^4$ dilution equivalent to 200 mIU/mL) or no or a low anti-HBc titer (HI titer $\leq 2^4$ dilution) is defined as seronegative. The combination of the above serologic screening tests excludes HBsAg-positive donors and most occult carrier donors. Because the cutoff point for HI is set at a higher level than that for enzyme immunoassay (EIA) anti-HBc, there are donations that are accepted while having low anti-HBc titer detectable by EIA but not by HI. Fifty seronegative samples are then pooled for NAT. JRC NAT screening employs a polymerase chain reaction (PCR) system (AmpliNAT, Roche, Indianapolis, IN) that utilizes multiplex reagents for detecting HBV, HCV, and HIV genomes. The detection range limit (95% confidence interval) for HBV is 22 to 60 copies per mL.[10] The sensitivity of current 50-NAT is expected to be 50-fold lower than that described above, that is, 1100 to 3000 copies per mL.

All donations from repeat donors received from 1997 to 2004 were subjected to a lookback study when a subsequent donation turned positive for a 50-NAT, HBsAg, or anti-HBc. Repository samples from donors immediately preceding donations that became positive for the presence of the markers described above were routinely analyzed by ID-NAT. Donors exhibiting a subsequent donation with anti-HBc reactivity above 2^4 dilution in the absence of anti-HBs, or donors with a decrease in their anti-HBs reactivity (to below 2^4 dilution) in the presence of a positive anti-HBc result, were also defined as anti-HBc converters and included in the study. The process of ID-NAT was as follows. Test samples were first screened with the same reagent and method as AmpliNAT except that the system contained a capture probe only for HBV. Positive samples were then subjected to dual-repeat ID-NAT. Doubly positive (+/+) and singly positive (+/–) samples were defined as ID-NAT–positive and further subjected to quantitative NAT. The HBV genome was quantified by a JRC in-house method that utilizes TBF-1 (nucleotides 250-272; 5′-AGACTCGTGGTGGACTTCTCT CA-3′), TBR-1 (nucleotides 428-409; 5′-TGAGGCATAGCA GCAGGATG-3′), and TP-02 (nucleotides 368-392; 5′-TATC GCTGGATGTGTCTGCGGCGTT-3′) as F-primer, R-primer, and probe, respectively. The sensitivity of the quantitative NAT was 100 copies per mL. Samples verified to be ID-NAT–positive were reevaluated for anti-HBc by EIA (AxSYM, Abbott Japan, Tokyo, Japan) as well as semiquantitative HI and for anti-HBs by EIA (AxSYM). Only samples exhibiting concordant results for anti-HBc with $2^1 \leq$ HI $\leq 2^4$ and EIA positivity (>50% inhibition) were defined as samples with low anti-HBc titers. To substantiate the infectious status of the donors in the ID-NAT-only–positive donation, repository tubes obtained in the subsequent donations were also evaluated for HI, EIA-anti-HBc, EIA-HBsAg, and EIA-anti-HBs.

The risk analysis for HBV contamination in blood components was limited to donations obtained from February 1, 2000, to January 31, 2004, all of which had been qualified by the current screening algorithm including 50-NAT. For the analysis of the infectivity of HBV-containing units, components that had not been tested by 50-NAT were also used in the study.

ID-NAT results for the preceding donation were sent with an evaluation form for viral contamination risk to the medical facility that used the donation. On the form, the degrees of viral contamination risk were described with the following classification: the donation was 1) ID-NAT–positive; 2) ID-NAT–negative but with probable HBV contamination because the blood was donated during the potential window period (the window period was defined as 46 days according to the Japanese guideline for lookback investigation issued by the Ministry of Health, Labour and Welfare); 3) ID-NAT–negative but with possible HBV contamination even though the time interval between the preceding donation and the marker conversion was long (>46 days); or 4) ID-NAT–negative and unlikely to have viral contamination because the test result for the subsequent donation was verified to be positive because of the alteration of the cutoff point for anti-HBc during the period between the two donations. The clinical outcome of patients transfused with ID-NAT–positive components was collected by local blood centers. The testing of blood samples from transfused patients, the initial diagnosis of HBV-TTI, and the report of suspected cases to blood centers were basically conducted by physicians who treated the patients. The diagnostic bases for TTI was therefore not uniform regarding the HBV markers tested, the observational period after index transfusion, and the time interval between transfusion and blood testing. In most medical institutions, the presence of anti-HBc or anti-HBs in pretransfusion samples is usually not evaluated. In all documented HBV transmission cases, the diagnosis of HBV infection was established on the basis of

TABLE 1. Numbers of ID-NAT–positive donations and positivities for anti-HBc in repository tubes

Converted markers	Projected number of converted donations for 4-year period	Number of repository tubes positive for ID-NAT within 4 years	Anti-HBc status of ID-NAT–positive repository sample		
			Low titer	Negative	Not tested
50-NAT	329	28 (8.5%)*	13	13	2
HBsAg†	730	16 (2.2%)*	3	13	0
Anti-HBc	14,662	114 (0.78%)*	79	34	1
Total	15,721 (observed number)	158 (1.01%; 39.5/year)	95 (60%)	60 (38%)	3 (2%)

* Percentage of observed ID-NAT–positive samples among projected number of samples for which ID-NAT was performed.
† HBsAg conversion includes those accompanied by anti-HBc seroconversion.

the HBsAg conversion, anti-HBs seroconversion, or HBV DNA conversion.

TABLE 2. Relationship between EIA-anti-HBs and HBV genomic copy number

EIA value for anti-HBs (mIU/mL)	Copies/mL (number of samples)
EIA = 0	<100 (14), 160, 200, 220, 230 (2), 300 (2), 380
0 < EIA < 5.0	<100 (11), 100, 120
EIA ≥ 5.0 (range, 5.7-179.2; median, 24.9)	<100 (18)

RESULTS

Lookback study for HBV

Of the repository tubes that had been aliquoted from the donations obtained from February 1, 2000, to January 31, 2004, a total of 15,721 were subjected to ID-NAT (Table 1). All the donations had been qualified with the current screening algorithm including 50-NAT. Although the total number of tubes investigated by ID-NAT is evident because ID-NAT was performed in one laboratory, the numbers for each of the HBsAg–, 50-NAT–, or anti-HBc–converted repeat donors are not available because of a data management problem where the detail of lookback process for each conversion case was not recorded on a centralized computer system. The exact number for each of the three categories was summed up only for the period between June 13, 2002, and July 21, 2003. From the extrapolation of these 404 days of data, the number of repeat donors who became positive for the presence of the three markers during the 4-year period was estimated (Table 1).

Of the estimated 329 prior donations that became positive for the presence of HBV DNA by 50-NAT in the subsequent donation, 28 (8.5%) were verified to be positive for the presence of HBV DNA by ID-NAT (Table 1). Viral concentrations were available for 18 samples and were fewer than 380 copies per mL. A low anti-HBc titer below the screening cutoff value ($\leq 2^4$ by HI test) was detected in 13 of 26 cases evaluated but not in the remaining 13 (Table 1). This result suggests that a significant number of occult carriers have a fluctuating low-level viremia. Thirteen of the 16 ID-NAT–positive donations in which subsequent donations became HBsAg-positive were thought to have been obtained during the window period because they were anti-HBc–negative (Table 1). The remaining three contained a low anti-HBc titer and were considered to be derived from chronic carriers. Seventy-nine of the 114 ID-NAT–positive donations exhibiting a subsequent donation with anti-HBc seroconversion were considered to have been derived from occult carriers having low anti-HBc titers (Table 1); 82 percent (65/79) of these carrier donors were more than 50 years of age at the time of the repository sample. Thirty-four donations with negative anti-HBc reactivity were thought to have been obtained during the window period of acute infection. These units were donated mostly by young people, with 68 percent (23/34) less than 50 years of age.

In summary, during the 4-year study, we identified 158 HBV ID-NAT-only–positive blood donations that corresponded to 1.01 percent of samples for which ID-NAT was performed. Ninety-five (60%) of them were donated by occult carriers with low-titer anti-HBc and 60 (38%) by anti-HBc–negative or window-period donors (Table 1). Thus, of the repeat donors in Japan, there are an estimated 39.5 donations every year that are ID-NAT-only–positive. Viral concentrations were generally very low in occult carrier donors with 75 (95%) of the 78 samples studied having fewer than 100 copies per mL and only three having at least 100 copies per mL (data not shown). Donations obtained during the window period contained higher viral DNA levels with 12 of the 46 samples studied containing at least 100 copies per mL (range, 100-860 copies/mL).

Anti-HBs titer was measured with EIA in 53 repository tubes for which clinical outcome as a result of transfusion were available (see below). When anti-HBs titer was divided into three categories (=0, between 0 and 5, and ≥5 mIU/mL), there was no sample that contained at least 100 copies per mL of HBV DNA in the category of anti-HBs of at least 5 mIU per mL (Table 2).

Infectivity of ID-NAT–positive component

To establish the infectivity of the ID-NAT-only–positive components, we included donations obtained from 1997

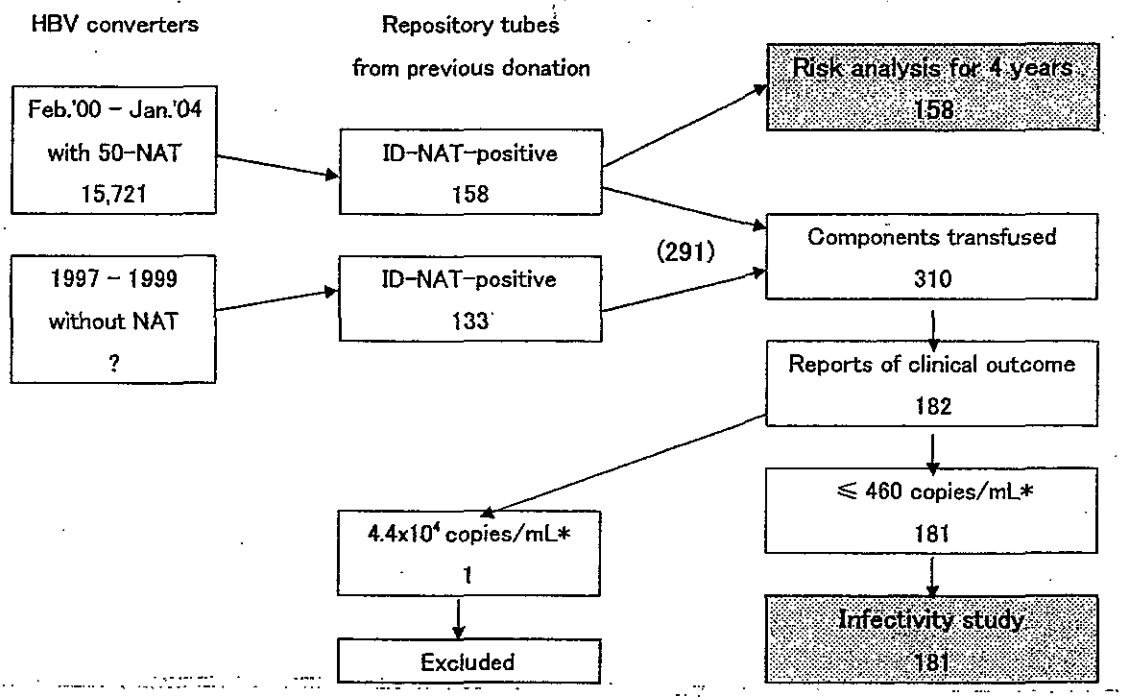

Fig. 1. Donations with HBV conversion obtained from 1997 to 2004. *HBV DNA copy number in repository tubes from presero-conversion donation.

to 2004 in the analysis (Fig. 1). A total of 291 donations were determined to be ID-NAT–positive and an estimated 310 components were prepared from these donations and distributed to medical institutions. Through the hemovigilance system, we received 182 reports as of August 2005 that showed the clinical outcomes of the transfusions of the components. Although the donations given before February 2000 were not screened by 50-NAT, it is highly unlikely that the ID-NAT–positive donations before February 2000 would have been disqualified by 50-NAT screening considering the very low viral load in these samples (≤460 copies/mL, except for one case). Therefore, they are also referred to in this article as ID-NAT-only–positive donations. One sample donated before NAT screening that contained 4.4×10^4 copies per mL HBV DNA was excluded from this study.

TABLE 3. Clinical outcome of 181 patients transfused with ID-NAT-only–positive components

Clinical outcome	Number of patients (n = 181)
Infected through transfusion	12
Positive for the presence of HBsAg	7
Positive for the presence of HBV DNA	2
Positive for the presence of anti-HBs	3
Positive after transfusion without pretransfusion testing	7
Infected before transfusion	7
No evidence of infection	51
Expired	104

Twelve of the 181 reports were confirmed to be HBV-TTI cases (Table 3); of these, 7 became positive for the presence of HBsAg, 2 became positive for the presence of HBV DNA, and 3 showed anti-HBs seroconversion. All these patients had negative results for either HBsAg or HBV DNA before transfusion. HBV DNA sequences were identical between the blood donors and the transfused patients in all the 8 pairs studied. Seven patients who were positive after transfusion for the presence of anti-HBc (2 patients), anti-HBs (3 patients), or both markers (2 patients) were not classified as TTI cases because of the lack of pretransfusion HBV testing data. The other 7 patients who were also positive for the presence of HBV markers after index transfusion were actually found to have anamnestic HBV infection before transfusion. Fifty-one patients showed no evidence of HBV infection after index transfusion. Because no anti-HBc testing was performed after transfusion in any of the 51 patients, we may have overlooked cases that showed positivity only for anti-HBc as a result of infection. A total of 104 patients had died without any test results recorded regarding HBV infection. Thus, 12 of 63 (of 12 infected and 51 noninfected) patients acquired HBV infection as a result of the transfusion of ID-NAT-only–positive components, suggesting the infectivity of the components to be 19 percent. If all of the 7 positive patients without pretransfusion data were

TABLE 4. Blood donors and components included in study of HBV transmissibility and relationship between HBV-TTI and anti-HBc status of transfused components

Anti-HBc in repository samples	Number of donors	Converted marker	Number of components processed and transfused	Total number of components evaluated	Infection	
					Yes	No evidence
Low titer	29	NAT: 4	5	33	1	32
		Anti-HBc: 25	28			
Negative	20	NAT: 1	1	22	11	11
		HBsAg: 4	4			
		Anti-HBc: 15	17			
Undetermined	6	NAT: 1	1	8	0	8
		Anti-HBc: 5	7			
Total	55	NAT: 6	7	63	12	51
		HBsAg: 4	4			
		Anti-HBc: 45	52			

assumed to be TTI cases, the infectivity of the components would be 27 percent.

Blood components transfused to the 63 patients had been processed from 55 donations (Table 4). The HBV infection status of the 55 donations was determined from the anti-HBc and anti-HBs data and was further confirmed by the serologic data that were obtained with the repository tubes in the subsequent donation when HBV marker(s) converted. Twenty-nine donors with low-titer anti-HBc were judged to be in the occult carrier state (Table 4); 4 donors who became 50-NAT–positive in the subsequent donation were anti-HBc–positive with greater than 95 percent EIA inhibition in both the index and the subsequent donations, and all 25 donors showed weak reactivity for HI and greater than 90 percent (except for one case with 63%) EIA inhibition in the index donation and showed HI positivity and greater than 95 percent EIA inhibition in the subsequent donation. One exceptional donor with 63 percent EIA inhibition for anti-HBc was a 60-year-old woman with 72 mIU per mL anti-HBs in the index donation. Twenty anti-HBc–negative donations were designated as those obtained during the window period (Table 4); 4 donors became strongly positive for EIA-HBsAg with greater than 200 S/N within 2 months; 13 donors became anti-HBc–positive with greater than 90 percent EIA inhibition coupled with HI conversion in the subsequent donations; 1 donor with 5 percent inhibition for EIA-anti-HBc became HI-positive with an increase of up to 65 percent inhibition and anti-HBs conversion; 1 donor with 10 percent inhibition became HI-positive although EIA-anti-HBc remained negative after 14 days with 46 and 38 percent inhibitions in duplicate tests; and 1 donor became positive only for 50-NAT after 33 days, representing a minipool (MP)-NAT window case. For the six donations that had conflicting results between HI and EIA-anti-HBc, the infection status was not determined even if the serologic data in the subsequent donation were taken into consideration. A nonspecific reaction of HI and EIA-anti-HBc or the recovery phase of acute infection with or without prior vaccination are the possible explanation for this category. Because the transfusion of components derived from one donation (e.g., red blood cells [RBCs] and fresh-frozen plasma [FFP]) sometimes caused discrepant results in patients and the total viral loads of the components were different depending on the component types, clinical outcomes resulting from the transfusion of components derived from single donation are described separately in the present analysis.

Note that 11 of the 12 components that caused HBV infection had zero or negligible anti-HBc titers and therefore were considered to have been derived from donations given during the 50-NAT window period (Table 4). One jumbo FFP component (450 mL) identified by 50-NAT conversion in the subsequent donation had low anti-HBc titer with fewer than 100 copies per mL viral concentration. This case represents a TTI case caused by the transfusion of an occult carrier–derived component. Results of anti-HBs testing were available for 9 of the 12 infectious samples (Table 5). All 9 showed negative results including the sample from the occult carrier that had 4.8 mIU per mL, a value defined as negative. Of the repository tubes from 51 noninfectious components, 11 were negative and 32 were positive with low titer for anti-HBc (Table 4). Of the 32 samples with low-titer anti-HBc, 11 were positive and 12 were negative for the presence of anti-HBs (Table 5).

In summary, of the 33 components with low-titer anti-HBc, only 1 could be identified that caused infection, whereas of the 22 anti-HBc–negative components, 11 had proved to cause infection (Table 5). If the 8 anti-HBc–undetermined cases were also considered, the infectivity range of low-titer anti-HBc components was 2.4 to 3.0 percent and that of anti-HBc–negative components was 37 to 50 percent, showing that the HBV transmission rate of window period–derived components observed in our hemovigilance system is more than 10-fold higher than the rate caused by occult carriers with low-titer anti-HBc

TABLE 5. Relationships between anti-HBc, anti-HBs, and HBV-TTI

Anti-HBc	Infection	Anti-HBs			Total
		Positive	Negative	Undetermined	
Low titer	Yes	0	1 (1)*	0	1
	No†	11 (17)	12 (15)	9 (0)	32
Negative	Yes	0	8 (9)	3 (2)	11
	No	0	8 (10)	3 (1)	11
Undetermined	Yes	0	0	0	0
	No	5 (5)	2 (2)	1 (1)	8
Total	Yes	0 (0)	9 (10)	3 (2)	12
	No	16 (22)	22 (27)	13 (2)	51

* Number in parentheses is the number of donations that include ones whose anti-HBs status was estimated with the data obtained from subsequent donation.
† No evidence of infection.

who have slipped through the JRC screening system. It is also clear that there was no HBV-TTI in patients who were transfused with anti-HBs–positive components. When anti-HBc–undetermined cases were included, none of 16 anti-HBs–positive components exhibited evidence of infection (Table 5). When anti-HBs–undetermined cases were reevaluated with the anti-HBs and/or anti-HBc data in the subsequent donation and time interval between the two donations, the cases were successfully divided into anti-HBs–positive or –negative groups (numbers in parenthesis in Table 5). When the estimated numbers were also taken into account, it is calculated that the infectivity of components with and without anti-HBs was 0 of 22 and 10 of 37, respectively (Table 5).

In both the low-anti-HBc-titer group and the anti-HBc–negative group, the same HBV DNA levels were measured in the components with and without evidence of infection (Table 6). This is true even when viral load is adjusted to total viral copy number contained in the components with mean plasma volume of each component (data not shown). There was also no clear correlation between the type of blood components transfused and the infection outcomes observed in the recipients. There were two cases where components from one donation caused discrepant results in two patients transfused (Table 6 footnote). The distribution of age or sex showed no difference between the patients with and without serologic evidence of infection (data not shown).

The basic disease conditions of the TTI cases were available for nine patients: multiple trauma in one, surgical operation in three, shock due to trauma and burn in one, great vessel diseases in two, hematologic malignancies in two, and disease unknown in three (Table 7). The results show that patients prone to infection were not limited to heavily immunocompromised people. Four patients showed elevated alanine aminotransferase (ALT) of more than 100 IU per L. Although the number of patients was limited, there was a tendency that a high ALT was associated with a high total viral load infused.

DISCUSSION

The sample repository of all donations stored since 1996 enabled us to perform a lookback study and identify the blood donations that were ID-NAT–positive with low HBV DNA levels but had not been detected by the regular 50-NAT, HBsAg, and anti-HBc screening tests. Although the serologic testing of pre- and posttransfusion blood samples of patients who had received the ID-NAT HBV DNA–reactive blood components was often incomplete, we were able to show a difference in the transmissibility of HBV between donations in the pre-HBsAg and/or MP-NAT window phase and blood from donors with occult HBV infection whose anti-HBc titers were below the exclusion limit of the JRC screening system.

We performed HBV ID-NAT on 15,721 repository tubes and identified 158 (1.01%) DNA-positive samples, of which 95 were anti-HBc–reactive with low titer, indicating that, under the Japanese screening algorithm, 60 percent of the ID-NAT-only–positive donations from repeat donors are derived from occult carriers. The viral loads of the samples from occult carriers were very low with 75 of 78 samples having fewer than 100 copies per mL. Such concentrations cannot be detected with pool-NAT systems[11] suggesting the need to develop and implement ID-NAT systems for screening.[12] When no ID-NAT systems are available, sensitive anti-HBc testing is currently the only measure for identifying these very low viral loads from occult carrier donations.[13,14]

Of the 63 patients transfused with ID-NAT-only–positive components, 12 (19%) were confirmed as HBV-TTI cases. This number is unexpectedly low relative to the high HBV infectivity observed in clinical settings and animal experiments.[15,16] Possible reasons for this include the following: 1) anti-HBs or anti-HBc testing before and after transfusion is not routinely performed in medical facilities; thus some patients might have immunity against reinfection from past exposure to HBV or vaccination, whereas a relatively large proportion of HBV infections may not have been recognized in the current hemoviligance system in the hospitals. It is, however, unlikely that most noninfected patients maintained immunity by past infection given the hepatitis marker frequency of first-time donors in Japan, where the highest prevalence of anti-HBc (6.82%) has been observed in donors in their sixties.[17] Assuming that this percentage represents the mean anti-HBc prevalence among patients who received transfusions and that all anti-HBc–positive people have immunity against reinfection, prior protection would have contributed very little to the observed incidence of HBV-TTI. 2) The viral copy number obtained

TABLE 6. Relationship between HBV infectivity, HBV viral loads, and types of components

Anti-HBc status	Infectivity	Viral loads (copies/mL); component type(s)
Low titer (33)	Infectious (1)*	<100; FFP (1)
	Without evidence of infection (32)	<100 (27), 93, 98, 100, 120, NT†; RBCs (17), FFP (14), PC‡ (1)
Negative (22)	Infectious (11)	<100 (6), 160, 170, 230§, 300‖, 380; RBCs(5), FFP(4), PC(2)
	Without evidence of infection (11)	<100 (8), 170, 230§, 300‖; RBCs (5), FFP (4), PC (2)
Undetermined (8)	Without evidence of infection (8)	<100 (6), 200, 460; RBCs (2), FFP (6)

* Number in parentheses is the number of components.
† NT = not tested.
‡ PC = platelet concentrate.
§ RBCs and FFP were processed from the donation and transfused to a patient undergoing orthopedic surgery and a gastric cancer patient, respectively. The former patient acquired HBV infection and the latter did not.
‖ FFP and RBCs were processed from the donation and transfused to a patient with dissecting aneurysm and a patient undergoing orthopedic surgery, respectively. The former patient acquired HBV infection and the latter did not.

TABLE 7. Basic disease conditions of TTI cases

Age (years)	Sex	Basic disease status	Converted marker	Highest ALT (IU/L)	Types of components transfused	Viral copies/mL in components	Total viral loads infused*
65	Male	Multiple trauma	HBsAg	2281	FFP-2 (WP)†	1.7×10^2	27,200
66	Female	Knee joint replacement (rheumatoid arthritis)	Anti-HBs	1109	RBCs-2 (WP)	2.3×10^2	5,750
49	Female	Dissecting aneurysm	HBsAg	992	FFP-2 (WP)	3.0×10^2	48,000
71	Male	Gastrectomy (gastric cancer)	HBsAg	148	RBCs-2 (WP)	<100	UD‡
62	Male	Acute myelogenous leukemia	HBV DNA	66	PC§-15 (WP)	3.8×10^2	95,000
63	Male	Carotid artery stenosis	HBsAg	56	RBCs-2 (WP)	<100	UD
74	Female	Shock due to trauma and burn	HBsAg	33	FFP-1 (WP)	<100	UD
68	Male	T-cell lymphoma	HBsAg	27	PC-10 (WP)	<100	UD
78	Male	Gastrectomy (gastric cancer)	HBV DNA	17	RBCs-2 (WP)	<100	UD

* Total viral load in the component was calculated with the plasma volume of each component; FFP-2, 160 mL; RBCs-2, 25 mL; PC-15, 250 mL; FFP-1, 80 mL; PC-10, 200 mL.
† Components obtained during the window phase (WP).
‡ UD = undetermined.
§ PC = platelet concentrate.

by PCR may include replication-incompetent DNA fragments and therefore may not directly relate to infectivity. 3) A protective effect may be induced by passively transferred anti-HBs from index components or components from other antibody-positive donors transfused close to the time of HBV exposure. 4) The infectivity of HBV may be reduced during storage of the blood components.

Despite these limitations we were able to compare the outcomes of HBV transmission of components derived from 55 donations and compare the infection outcomes in 63 recipients. The results that were retrievable in the hemoviligance system indicate that the donations in the pre-HBsAg and/or MP-NAT window phases are at least 10-fold more infectious than the donations with low anti-HBc titers from occult carriers. Eleven of 22 (50%) components derived from window-phase donations proved to have caused seroconversion of HBV markers in the recipients, whereas only 1 of 33 (3%) anti-HBc–reactive donations showed serologic evidence of infection. The infectious anti-HBc–reactive FFP component came from an anti-HBs–negative occult HBV carrier with fluctuating viremia. None of 11 anti-HBc–reactive and anti-HBs–positive units proved to have caused infection. When the anti-HBc–undetermined units were taken into consideration, it was revealed that there was no evidence of infection in any of the 16 patients transfused with anti-HBs–positive components. To establish the relationship between the titer of anti-HBs and the infectivity of occult carrier–derived blood, more clinical reports are needed for the category of patients who acquired infection as a result of transfusion of low titer anti-HBc components. When the clinically observed HBV transmission rate of anti-HBs–negative donations with and without low-titer anti-HBc was compared, it turned out that the transmission rate caused by the presumed tail-end carriers with occult HBV infection (1/13, 8%) was still significantly lower than the rate of the window-phase donations (8/16, 50%; $p < 0.05$). There were three components having at least 200 copies per mL of HBV sequence that did not cause infection even in the absence of detectable anti-HBs. It may be that the infectivity of donors with higher anti-HBc titers (that are screened out of the Japanese blood supply) have a higher risk to be infectious, particularly in the tail-end chronic carriers without circulating

neutralizing anti-HBs. More research needs to be done to understand the infectivity of HBV in donors with chronic occult infection or with persistent or recurrent viral replication after recovery. In chronic and perhaps more in recovered occult HBV infection, mutations in the genome may reduce the capacity of viral entry, replication, and secretion.[18-24] It has been reported that a portion of patients who recovered from acute hepatitis harbor HBV in their liver and may exhibit intermittent viremia even after complete clinical resolution and HBsAg clearance.[25-28]

There was essentially no difference in copy number between the infectious and the presumed noninfectious components, indicating that the viral load is not the only factor for infectivity. The infectious load is dependent on the amount of plasma and 3- to 20-fold higher in platelet concentrate or FFP than in RBCs, but we were unable to demonstrate a difference in transmission risk between these components. It may be that other factors affecting the observed infectivity in recipients (see above) have masked the known relationship between infectivity and viral load in the transfused components. Unfortunately we have no means to confirm whether indeed half of the anti-HBs–negative window-phase units were not infectious even though viral loads above 10^2 to 10^5 HBV particles have been infused. It may be that we would have found these donations to be infectious when anti-HBc and anti-HBs was routinely tested in the pre- and posttransfusion samples of the recipients. Our estimates for the infectivity of ID-NAT-only–positive units may thus be underestimated. We also could not find any clear differences between the patients' susceptibility profiles in terms of disease status, age, and sex. Although we could not find clear relationship between infectivity and viral load in components, some degree of association was suggested between patients' high ALT value during the course of infection and the high total viral load contained in the component, the result reminiscent of the report by Barker and Murray.[29]

JRC has been collecting voluntary reports on TTI from hospitals and has already established a database for isolated TTI cases.[30] Combining these data and those obtained from this lookback study, it is estimated that the total number of HBV-TTI cases is 17 to 20 per year (1/0.27-0.32 million donations) in Japan with 5.4 million annual blood donations and that approximately 85 percent of the HBV infections are caused by the transfusion of window period–derived components. Although it is obvious that combined screening with ID-NAT and sensitive anti-HBc testing would ultimately reduce the HBV-TTI cases, a decision on whether the anti-HBc screening is implemented or not should be made considering the local prevalence of HBV in the area considered, the number of donors who would be disqualified as a result of anti-HBc screening, and the relative low infectivity of occult carrier–derived components described in this report.[31] One must bear in mind, however, that in our study only the infectivity of low-titer anti-HBc carriers was examined and that the picture can be completely different for anti-HBs–negative occult carriers with higher anti-HBc titers. It should also be mentioned that JRC's serologic test, especially hemagglutination for HBsAg, has lower sensitivity than EIA and that the rate of screening NAT yield and ID-NAT-only–positive donation can be different in other areas.

Inaba and coworkers[32] recently reported about another anti-HBs–negative occult carrier with borderline detectable anti-HBc levels who was found some of the time to be ID-NAT–positive and some of the time to be ID-NAT–negative.[32] This may be caused by fluctuating viremia in the donor or stochastic sample variability in the NAT assay typical with low viral load. Lookback study showed that some of the previous donations, both ID-NAT–positive and ID-NAT–negative units, had caused HBV infection in the recipients and that some had caused clinical hepatitis. This donor only would have been detected with more sensitive anti-HBc screening or with ultrasensitive ID-NAT. JRC is currently exploring which options remain to further reduce the risk of posttransfusion hepatitis B.

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医薬品 研究報告 調査報告

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 20	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)		前橋美智子, 高瀬隆義, 水戸瀬利行, 松崎哲夫, 桐林克己, 田中榮作, 崎山樹, 岡部雅弘. 第31回日本血液事業学会総会; 2007 Oct 3-5; 高松.	日本

研究報告の概要

○献血者への追跡調査(献血後情報)により輸血によるHBV感染が示唆された一症例について

はじめに:平成19年3月、A医療機関より輸血によるHBV感染が疑われるとの報告が千葉県赤十字血液センターにあった。因果関係の確認のために実施した当該輸血用血液製剤に係る保管検体個別NATは陰性であり、献血者追跡調査により輸血によるHBV感染が示唆された一症例を経験したので報告する。

症例:患者は70歳代女性、平成18年9月に人工関節置換術の際に、今回の当該輸血用血液製剤(RC-M・A・P2単位)を含む4単位の輸血を受けた。輸血前のHBs抗原は陰性。平成19年3月上旬、食欲不振、全身倦怠感、ビリルビン尿出現により近医を受診。肝機能障害、黄疸症状が認められ、輸血を実施したA医療機関に紹介され入院となった。入院時の血液検査では、HBs抗原陽性、HBc抗体(IgM)陽性、AST、ALTは高値であり急性B型肝炎と診断された。A医療機関より副作用感染症報告を受け、当該輸血用血液製剤に係る保管検体2本について、個別NATを実施したが2本とも陰性であった。A医療機関担当医から、患者の感染経路は輸血以外に考えにくく、献血者の追跡調査の要望があった。当該輸血用血液製剤に係る献血者2名には、その後の献血歴はなく、血液センターからのフォローアップの結果、1名の献血者については平成19年1月にB型肝炎を発症し、B医療機関で治療中であるとの情報が寄せられた。献血者とB医療機関の了解を得て、平成19年4月受診時の検体を検査したところHBV-DNAが検出され、患者のそれと塩基配列が一致した。他の1名の献血者は、平成19年4月採血の追跡調査結果はHBV-DNA陰性であった。

まとめ:20プールNAT陰性、HBV保管検体個別NAT陰性であったが、献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された一症例を経験した。原因血はHBV感染のごく初期であったと推測された。

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	今後の対応
20プールNAT陰性、HBV保管検体個別NAT陰性であったが、献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された一症例を経験したとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)の導入を予定している。NATの精度向上についても評価・検討している。

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献血者への追跡調査（献血後情報）により輸血によるHBV感染が示唆された一症例について

千葉県赤十字血液センター[1]
日本医科大学付属千葉北総病院[2]
前橋美智子[1]，高瀬隆義[1]，水戸瀬利行[1]，
松崎哲夫[1]，桐林克己[1]，田中栄作[1]，崎山　樹[1]，
岡部雅弘[2]

【はじめに】平成19年3月，A医療機関より輸血によるHBV感染が疑われるとの報告が血液センターにあった。因果関係の確認のために実施した当該輸血用血液製剤に係る保管検体個別NATは陰性であり，献血者追跡調査により輸血によるHBV感染が示唆された一症例を経験したので報告する。

【症例】患者は70歳代女性，平成18年9月に人工関節置換術の際に，今回の当該輸血用血液製剤（RC-M・A・P2単位）を含む4単位の輸血を受けた。輸血前のHBs抗原は陰性。

平成19年3月上旬，食欲不振，全身倦怠感，ビリルビン尿出現により近医を受診。肝機能障害，黄疸症状が認められ，輸血を実施したA医療機関に紹介され入院となった。入院時の血液検査では，HBs抗原陽性，HBc抗体（IgM）陽性，AST，ALTは高値であり急性B型肝炎と診断された。A医療機関より副作用感染症報告を受け，当該輸血用血液製剤に係る保管検体2本について，個別NATを実施したが2本とも陰性であった。

A医療機関担当医から，患者の感染経路は輸血以外に考えにくく，献血者の追跡調査の要望があった。

当該輸血用血液製剤に係る献血者2名には，その後の献血歴はなく，血液センターからのフォローアップの結果，1名の献血者については平成19年1月にB型肝炎を発症し，B医療機関で治療中であるとの情報が寄せられた。献血者とB医療機関の了解を得て，平成19年4月受診時の検体を検査したところHBV-DNAが検出され，患者のそれと塩基配列が一致した。

他の1名の献血者は，平成19年4月採血の追跡調査結果はHBV-DNA陰性であった。

【まとめ】20プールNAT陰性，HBV保管検体個別NAT陰性であったが，献血者追跡調査により輸血用血液製剤からのHBV感染が示唆された一症例を経験した。原因血はHBV感染のごく初期であったと推測された。

医薬品 研究報告 調査報告書

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研究報告の概要

○NATスクリーニングのプールサイズ縮小による効果と分析

はじめに:輸血用血液製剤の安全性の向上を目的として、2004年8月よりNATスクリーニングのプールサイズを50から20に縮小した。今回、大阪府赤十字血液センターで検出されたHBV-NAT陽性事例を基にプールサイズ縮小の効果等について解析を行ったので報告する。

対象:大阪センターにおいて50プール及び20プールで検出されたHBV-NAT陽性事例の合計81人を対象とした。

結果:HBV-NAT陽性数は右表の通り。HBc抗体陽性の12人中、11人は50～60歳代でウイルス量が1,000コピー未満/mLであり、残りの1人は28歳でウイルス量が 1.7×10^5 コピー/mLであった。また、12人中10人の献血者について医師による面談及びフォローアップを行ったところ、急性肝炎の発症等に関する申告はなく、来所時の検査結果によるウイルスコピー数及びウイルスマーカーの変動はなかった。

考察:①プールサイズ縮小後に100コピー未満/mLのHBV-NAT陽性者の比率が高くなっていることから、縮小による効果があると思われた。②追跡調査、遡及調査及び医師の面談等による総合的な解析によりHBV低濃度キャリアが疑われる献血者がプールサイズ縮小後に多く検出されていることが推察された。

		50プール	20プール
陽性者		55人(2.72)	26人(2.50)
HBc抗体(EIA法)	陽性者	3人:5.5%(0.14)	9人:34.6%(0.86)
	陰性者	52人:94.5%(2.57)	17人:65.4%(1.63)
100コピー未満/mLの陽性者		1人:1.8%(0.04)	6人:23.1%(0.57)
1,000コピー未満/mLの陽性者		14人:25.5%(0.69)	8人:30.8%(0.77)
50～60歳代の陽性者		4人:7.3%(0.19)	10人:38.5%(0.96)

( )内は10万人当たりの本数

使用上の注意記載状況・その他参考事項等

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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

献血血液のNATスクリーニングのプールサイズを50から20に縮小した後、100コピー未満/mLのHBV-NAT陽性者の比率が高くなったことは縮小の効果と考えられ、HBV低濃度キャリアが疑われる献血者はプールサイズ縮小後に多く検出されたとの報告である。

今後の対応

日本赤十字社では、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。HBV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

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NATスクリーニングのプールサイズ縮小による効果と解析

大阪府赤十字血液センター

入江奥利子，岡　暗美，押野正次，堀江真理子，福森泰雄，中出　亮，平山文也，吉村敬次，谷　慶彦，柴田弘俊

【はじめに】輸血用血液製剤の安全性の向上を目的として，2004年8月よりNATスクリーニングのプールサイズを50から20に縮小した。今回，大阪センターで検出されたHBV-NAT陽性事例を基にプールサイズ縮小の効果等について解析を行ったので報告する。

【対象】大阪センターにおいて50プール及び20プールで検出されたHBV-NAT陽性事例の合計81人を対象とした。

【結果】HBV-NAT陽性数

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HBc抗体（EIA法）	陽性者	3人：5.5%（0.14）	9人：34.6%（0.86）
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100コピー未満/mLの陽性者		1人：1.8%（0.04）	6人：23.1%（0.57）
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50～60歳代の陽性者		4人：7.3%（0.19）	10人：38.5%（0.96）

（　）内は10万人あたりの本数

HBc抗体陽性の12人中，11人は50～60歳代でウイルス量が1,000未満コピー/mLであり，残りの1人は28歳でウイルス量が1.7×10^5コピー/mLであった。また，12人中10人の献血者について医師による面談及びフォローアップを行ったところ，急性肝炎の発症等に関する申告はなく，来所時の検査結果によるウイルスコピー数及びウイルスマーカーの変動はなかった。

【考察】①プールサイズ縮小後に100コピー未満/mLのHBV-NAT陽性者の比率が高くなっていることから，縮小による効果があると思われた。②追跡調査，遡及調査及び医師の面談等による総合的な解析によりHBV低濃度キャリアが疑われる献血者がプールサイズ縮小後に多く検出していることが推察された。

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-7

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 8 月 10 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			研究報告の公表状況	Evaluating the impact of public health notification of suspected transfusion- transmissible hepatitis C virus infection and effectiveness of lookback and traceback investigations by Canadian Blood Services in British Columbia, Canada. August 2002 through February 2005 Whitlock, M. et al. Transfusion, 47; 1534-1539 (2007)	公表国 カナダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
輸血伝播性 C 型肝炎ウイルス（TT-HCV）が疑われた症例はブリティッシュコロンビア州公衆衛生局（PH）への報告が求められ，2002 年 8 月より，その規制が施行された。本調査では，規制施行後 2 年半の TT-HCV 感染疑い例の増加数を調査するとともに，感染レシピエントにおける遡及（LB）調査及び感染ドナーにおける追跡（TB）調査の有効性を検討した。LB 調査は TB 調査により感染ドナーが特定された後に開始し，TB 調査は LB 調査により感染レシピエントが特定された後に開始した。PH への報告を介して特定された因果関係の否定できない TT-HCV 感染は 1%未満であることがわかった。全調査の 92%は 1992 年の第 2 世代抗 HCV 酵素免疫測定法によるドナースクリーニングを導入する前の輸血に関与していた。レトロスペクティブに TT-HCV 感染を特定するにあたって，スクリーニング導入日以降の輸血に対する LB 及び TB 調査実施の有益性はわずかであると考えられる。一方，HCV に感染したヒトの約 30%が LB 又は TB 調査の結果により，初めて自身の HCV の罹患状況を知ることとなった。結論として，現在の TT-HCV 感染疑いの報告手順では，その後の LB 調査又は TB 調査の開始までに長期間を要することが判明した。	BYL-2007-0292

報告企業の意見	今後の対応
本文献は，PH に報告される輸血を介した HCV 感染の疑いのある症例の現在の報告手順は効果がないということを明確に示唆している。対照的に，感染者数の確認には，第 2 世代抗 HCV 酵素免疫測定法によるドナースクリーニングの導入が極めて効果的である。HCV に対する NAT スクリーニングは，弊社の血漿分画製剤，コージネイト FS 又はコージネイト FS バイオセットの製造工程培地に使用されている血漿分画成分の製造工程における血漿プールで，体系的に実施されている。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

Evaluating the impact of public health notification of suspected transfusion-transmissible hepatitis C virus infection and effectiveness of lookback and traceback investigations by Canadian Blood Services in British Columbia, Canada, August 2002 through February 2005

Mandy Whitlock, Sandra Lord, Jane A. Buxton, Patrick Doyle, and Mark Bigham

BACKGROUND: Suspected transfusion-transmissible infections (TTIs) have been reported to public health (PH) in British Columbia (BC) since August 2002. The impact of PH notification of suspected transfusion-transmissible hepatitis C virus (TT-HCV) infection over the first 2.5 years and the effectiveness of HCV lookback (LB) and traceback (TB) investigations conducted by Canadian Blood Services (CBS) in BC were evaluated.

STUDY DESIGN AND METHODS: Suspected TT-HCV cases reported to CBS in BC between August 28, 2002, and February 28, 2005, were analyzed. The incremental yield of plausible TTIs from PH-reported suspected TTIs was calculated. The effectiveness of LB and TB investigations was assessed with respect to the impact of improved anti-HCV donor screening, the number of newly recognized HCV infections, and the timeliness of initiating investigations.

RESULTS: Nine of 553 (1.6%) investigations were initiated after PH reporting, yielding an additional 2 of 237 (i.e., 0.8%) plausible TTIs. Ninety-two percent of investigations with transfused units involved transfusions before implementing second-generation anti-HCV enzyme immunoassay (EIA) donor screening. Almost one-third of HCV-infected persons in linked investigations (i.e., LB triggered by a TB and vice versa) were newly identified. Recently tested, PH-reported cases incurred a mean delay exceeding 6 months until initiating a LB or TB investigation.

CONCLUSION: PH reporting of TTIs and investigating transfusions after second-generation anti-HCV EIA donor screening identified few plausible TT-HCV infections. Many HCV-infected recipients or lapsed donors first became aware of their infection status as a result of CBS investigations. The current process of reporting suspected TTIs incurs significant time delay.

Hepatitis C virus (HCV) disease has been notifiable to public health (PH) in British Columbia (BC) since 1992 and since then, more than 55,000 cases have been reported (J.A. Buxton, BC Center for Disease Control, personal communication, December 2006). In many instances, epidemiologic follow-up information is lacking, resulting in underrecognition of suspected transfusion transmissible HCV infection (TT-HCV) to blood suppliers. Even when recognized, suspected TT-HCV may be inconsistently reported to agencies such as blood suppliers for further investigation. Therefore, a regulatory requirement to notify PH of suspected TTIs might be expected to increase the sensitivity of TT-HCV case detection through lookback (LB; i.e., notifying and testing recipients of potentially contaminated blood) or, reciprocally, traceback (TB) investigations

In 2000, suspected TTIs, including suspected TT-HCV, were made reportable to PH in BC under the provincial Health Act.[1] The regulatory change was implemented in

ABBREVIATIONS: CBS = Canadian Blood Services; LB = lookback; PH = public health; TB = traceback; TT-HCV = transfusion-transmissible hepatitis C virus; TTI(s) = transfusion-transmissible infection(s).

From the British Columbia Center for Disease Control, Canadian Blood Services, BC & Yukon Center, Vancouver Hospital and Health Sciences Center, and the Department of Health Care and Epidemiology, University of British Columbia, Vancouver, British Columbia, Canada.

Address reprint requests to: Dr Mark Bigham, Canadian Blood Services, BC & Yukon Center, 4750 Oak Street, Vancouver, BC, Canada V6H 2N9; e-mail: mark.bigham@bloodservices.ca.

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TRANSFUSION 2007;47:1534-1539.

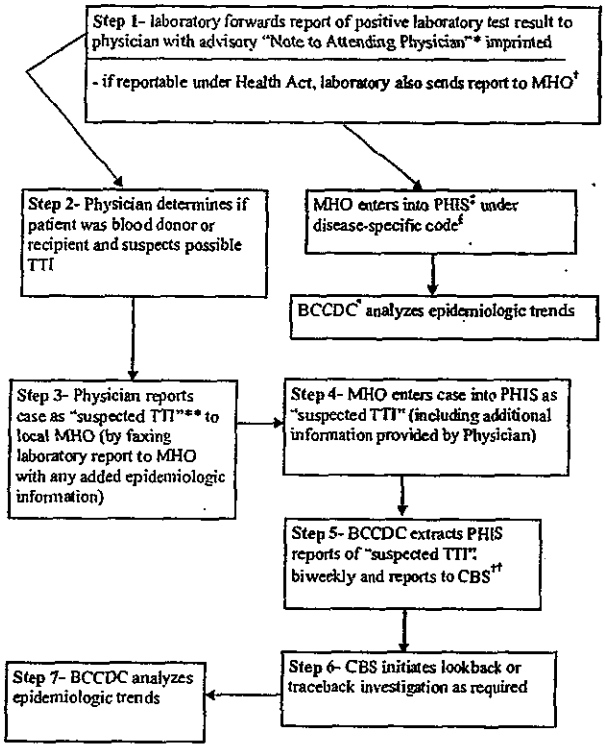

Fig. 1. Procedure in BC for reporting and investigating suspected TTIs. *Note to attending physician—a standardized advisory note printed on all laboratory reports with a positive laboratory test result for specified blood-borne pathogens, advising physician to report as suspected TTI if patient has donated or received blood. †MHO = Medical Health Officer; ‡PHIS = Public Health Information System (electronic provincial case registry for diseases reportable to PH); §disease-specific code, for example, HCV, HBV; ¶BCCDC = British Columbia Center for Disease Control (provincial PH laboratory); **TTI = transfusion transmissible infection; ††CBS = Canadian Blood Services.

August 2002, at which time reporting of suspect TTIs to PH began.[1] A suspected TTI was defined as a laboratory-confirmed, blood-borne infection diagnosed in a recipient of blood or blood products which, in their doctor's assessment, was likely transfusion transmitted.[2] Newly diagnosed cases of a blood-borne infection in a former blood donor were also included in the new reporting provisions. To our knowledge, BC is the first jurisdiction in the world to legally mandate reporting of suspected TTIs to PH. PH in turn forwards reports to Canadian Blood Services (CBS). In BC, CBS is responsible for coordinating and conducting all LB and TB investigations. The current procedure in BC for reporting suspected TTIs is shown in Fig. 1.

One objective of this statutory requirement for reporting suspected TTIs was to improve surveillance of the risk of TTIs such as TT-HCV.[2] Blood suppliers in Canada, the United States, and other countries, however, as well as hospitals in some jurisdictions, had already implemented targeted HCV LB and TB programs.[3-9] Evaluations of both general and targeted HCV LB investigations have reported relatively low yield in identifying HCV infected recipients.[8-15] A recent review of the Canadian experience with targeted HCV LB investigations[16] highlighted a trend, previously reported,[17-20] of further decreasing yield of such investigations since implementing increasingly sensitive serologic (i.e., second-generation[21] and later anti-HCV assays[22,23]) and nucleic acid–based[24,25] donor screening.

In this study, our primary objective was to quantify the incremental yield of identified TT-HCV infections, 2.5 years after implementing the regulatory requirement in BC to report cases of suspected TT-HCV to PH. Two secondary objectives were, first, to assess the effectiveness of HCV LB and TB investigations, not only in identifying previously unrecognized TT-HCV infections, but also in light of increasingly sensitive HCV donor screening, starting with implementation of second-generation anti-HCV assays, and second, to assess the timeliness of the current reporting process for suspected TTIs.

MATERIALS AND METHODS

Data from all cases of suspected TT-HCV (including co-infections with HCV and one or more other blood-borne pathogens) reported to CBS in BC, from August 28, 2002, to February 28, 2005, were entered into a computer database (Access, Microsoft Corp., Bellevue, WA) and imported into another computer program (Excel, Microsoft Corp.) for descriptive analyses. Cases were categorized as either "PH-reported" (i.e., reported to CBS through the new regulatory process for PH reporting of suspected TTIs) or as "non–PH-reported" (i.e., reported directly to CBS from all other sources, including physicians, hospitals, donors, or recipients). TT-HCV comprised 97 percent of all suspected TTI cases reported to CBS over this period. Outcome data from ensuing HCV LB and TB investigations were analyzed, including donor and recipient HCV test assay (e.g., enzyme immunoassay [EIA] or nucleic acid test), test date, and test result.

The plausibility of TT-HCV infections was determined according to an appropriate chronology of donor and recipient test results. Plausible TT-HCV infection was discounted in two circumstances: a positive recipient HCV test result before the date of transfusion in question in a HCV LB and, as specified by CBS procedures, a negative donor anti-HCV (second generation or later anti-HCV assay) test result from a specimen collected at least 140 days after the donation date of the transfusion in question (which cleared the donor from a HCV TB investigation). The impact of implementing PH reporting of

suspected TT-HCV infections was measured by comparing the yield of plausible TT-HCV infections from LB and TB investigations initiated as a result of PH-reported and non–PH-reported cases.

To gauge the impact of increasingly sensitive donor HCV testing, the proportions of HCV-infected donors and recipients identified in TB and LB investigations, respectively, were calculated for transfusions occurring before, compared with after implementing second generation anti-HCV donor screening. Second-generation anti-HCV EIA (Ortho Diagnostic Systems, Raritan, NJ) blood donor screening was implemented in Canada in March 1992.

To estimate the number of HCV infections in donors and recipients which was first recognized as a result of these investigations, we examined the outcomes of "linked" LB (i.e., an anti-HCV–positive donor first identified through a TB investigation) and "linked" TB investigations (i.e., an anti-HCV–positive recipient first identified through a LB investigation). For linked LB investigations, we examined the time from a donor's positive test result to the opening date of a LB investigation. If this time period was less than 100 days, then it was considered likely that the donor became aware of their infection status through CBS notification. Analogously, for linked TB investigations, we examined the time from a recipient's positive test result to the opening of a TB investigation as an indirect means of assessing whether a recipient likely became aware of their infection status through CBS notification.

To assess the timeliness of the current process for reporting suspected TTIs, we calculated the mean number of days from a positive HCV test result for a donor or recipient, to the opening of a LB or TB investigation, respectively, for PH-reported and non–PH-reported cases.

RESULTS

Yield of plausible TT-HCV infections from LB and TB investigations

During the study period, 31 suspected TT-HCV cases (including 2 cases with a history of both donation and receipt of blood) were reported to CBS from PH. Nine investigations (5 LB and 4 TB) were initiated as a result of PH-reported cases (Table 1). Fourteen other investigations also involving PH-reported cases had already been initiated as a result of prior (i.e., duplicate) reporting of cases to CBS from other sources.

Two plausible TT-HCV infections (one each from LB and TB investigations) were identified as a result of cases exclusively reported to CBS by PH (Table 2). By comparison, 235 plausible TT-HCV infections (127 from LB and 108 from TB investigations) were identified through reports received by CBS from non-PH sources (including 14 cases involving duplicate reporting from PH).

TABLE 1. Case reporting sources for HCV LB and TB investigations

	Case reporting source			
HCV investigation	PH	Non-PH	Duplicate*	Total
LB	5	232	10	247
TB	4	298	4	306
Total	9	530	14	553

* Case reported from both PH and non-PH sources.

Impact of second-generation anti-HCV EIA donor screening

A total of 122 of 128 (95.3%) HCV LB investigations with transfused units identified involved donations before second-generation anti-HCV EIA donor screening and 127 of 150 (84.7%) recipients from these earlier HCV LB investigations were anti-HCV–positive (Table 3). In contrast, only one anti-HCV–positive recipient was identified who was transfused after this time, and this recipient had also received 4 units implicated in HCV LB investigations before second-generation anti-HCV EIA donor screening. Similarly, for HCV TB investigations with transfused units identified, 153 of 171 (89.4%) involved donations before second-generation anti-HCV EIA donor screening. One hundred twenty-three of 406 (30.3%) donors from these earlier HCV TB investigations were anti-HCV positive, compared with none after this time.

Identifying previously unrecognized HCV infection

Eighty-five of 247 (34.4%) HCV LB investigations were initiated after identifying an infected donor through a TB investigation. Follow-up test results were available for 77 of these 85 donors, of whom 29 (37.7%) first learned of their HCV disease status as a result of the TB investigation (data not shown). Ninety-five of 307 (30.9%) HCV-TB investigations were initiated after identifying an infected recipient through a LB investigation, of whom 26 recipients (27.4%) first learned of their HCV disease status as a result of the LB investigation (data not shown).

Timeliness of initiating HCV LB and TB investigations

The overall mean number of days from a donor positive test result to initiation of a LB or TB investigation for both exclusively PH or non–PH-reported cases was similar and protracted—greater than 6 months (Table 4). The mean interval was reduced to 39.5 days after discounting one significant outlier among the five PH-prompted LB investigations (data not shown). Similarly, discounting one outlier among the four TB investigations involving PH-reported cases reduced the mean interval to 161 days (data not shown).

TABLE 2. Plausible TT-HCV infections identified through LB and TB investigations

Investigations	Case reporting source: PH	Non-PH (including duplicate*) reported	Total
HCV LB			
Investigations with transfused units	1	127	128
Units with recipient HCV test result available	1	155	156
Plausible TT-HCV infections	1	127	128
HCV TB			
Investigations with transfused units	1	170	171
Units with donor HCV test result available	1	529	530
Plausible TT-HCV infections	1	108	109

* Case reported from both PH and non-PH sources.

TABLE 3. Relative yield of TB and LB investigations before and/or after second-generation anti-HCV EIA donor screening

Investigations	Transfusion date: Before second-generation anti-HCV EIA	After second-generation anti-HCV EIA	Total
HCV LB			
Investigations with transfused units	122	7	129*
Number of available recipient HCV test results	150	6	156
Number of anti-HCV–positive recipients	127	1†	128
HCV TB			
Investigations with transfused units	153	23	176‡
Number of available donor HCV test results	406	124	530
Number of anti-HCV–positive donors	123	0	123

* One HCV LB investigation involved donations before and after second-generation anti-HCV EIA testing.
† Recipient received 5 units involved in HCV LB investigations, 4 of which were transfused before second-generation anti-HCV EIA testing.
‡ Five recipients received units both before and after second-generation anti-HCV EIA testing.

DISCUSSION

In the first 30 months since implementing TTI reporting in BC, 9 HCV LB or TB investigations were initiated following suspected TT-HCV cases reported exclusively by PH, comprising 1.6 percent of the 553 investigations that were opened during this period. From these 9 investigations, an additional two plausible TTIs were identified, representing an incremental yield of less than 1 percent (2 of 237) of plausible TTIs identified. Because unexplored alternative epidemiologic risk factors in these cases may also be more relevant, this analysis clearly indicates limited incremental benefit attributable to PH notification of suspected TTIs in BC.

Increasingly sensitive donor screening of HCV infection[17,21-25] has substantially reduced the risk of TTI-HCV.[11,16,18,19] In this study, 92 percent (275 of 299 [excluding 6 investigations involving one donor and five recipients who, respectively, donated or received units both before and after implementing second-generation anti-HCV EIA donor screening]) of LB and TB investigations (with transfused units) involved transfusions before implementing second-generation anti-HCV EIA donor screening in 1992 (Table 3). For transfusions since 1992, the incremental benefit of LB or TB investigations in identifying previously unrecognized TT-HCV appears marginal and supports the call by others[15,16,20] for a review of the medical, ethical, political, and legal rationale for undertaking such investigations involving recently transfused units.

In contrast, our evaluation of the PH impact of transmissible disease notification through LB and TB investigations identified a significant number of HCV-infected recipients and past donors who were likely unaware of their disease status. Over one-quarter (27.4%) of anti-HCV–positive recipients in LB investigations linked to a prior TB investigation first became aware of their infection through CBS notification, whereas for anti-HCV–positive donors in TB investigations linked to a prior LB investigation the corresponding proportion was 37.7 percent. This is somewhat lower than the range (42%-68%) of newly identified HCV infections from general or targeted HCV LB investigations reported from earlier investigations undertaken in Canada and the United States[3,4,6,10,11] and could reflect a diminishing proportion of cases associated with transfusions before the advent of donor anti-HCV testing. As pointed out by AuBuchon,[15] however, the health benefit of earlier detection of TT-HCV is likely mitigated by the reduced cost-effectiveness (i.e., cost per life-year gained) of HCV treatment for many HCV-infected blood recipients, given a mean transfusion recipient age of 60 to 65 years.[26] With passing time, it can be confidently predicted that HCV LB and TB investigations will detect an ever-decreasing proportion of previously unrecognized HCV infection.

This study identified prolonged intervals between the date of a positive laboratory test result in a blood donor or recipient and opening of an appropriate LB or TB investigation. Minimizing these times may enable potentially

TABLE 4. Interval (days) between positive HCV test result and initiation of HCV LB or TB investigation

	Days from positive HCV test result to initiating HCV LB or TB investigation							
	PH reported cases				Non-PH (including duplicate*) reported cases			
Investigations	Number	Mean	Median	Range	Number	Mean	Median	Range
HCV LB	5	232	52	2-1004	232	799	89	2-4353
HCV TB	4	321	221	41-801	296	1427	1020	7-4880

* Case reported from both PH and non-PH sources.

infectious in-date blood products—primarily fresh-frozen plasma—to be withdrawn from inventory sooner. Although it appears that PH-reported cases were investigated sooner, this is largely an artifact of different time periods for laboratory test dates between PH and non-PH cases. All investigations of PH-reported cases during the study period were as a result of laboratory testing that had been performed between 2000 and 2005, whereas investigation of non–PH-reported cases that were initiated during the study period had laboratory testing performed as far back as 1990.

Measures have been implemented in BC to reduce the reporting delay of suspected TTIs, such as establishing a provincial Central Transfusion Registry in 1998. The current process for reporting suspect TT-HCV continues, however, to incur prolonged delay for several reasons. First, prior donation or transfusion history may be inconsistently or unreliably recalled.[9,27-29] Second, infections with reporting case definitions that require manual integration of both laboratory and clinical data are more likely to be underreported and reported in a less timely fashion than routine, automated electronic laboratory reporting of positive laboratory results.[27-31] To improve the efficiency of identifying and reporting suspected TTIs to CBS, an automated, anonymized data linkage process between the provincial PH laboratory at BCCDC, CBS, and the provincial Central Transfusion Registry, is currently being evaluated.[32]

In summary, less than 1 percent of plausible TT-HCV cases were identified exclusively through PH reporting, along with a similar low yield of infected cases identified from investigations involving transfusions after second generation anti-HCV donor screening. Overall, approximately one in three suspected TT-HCV infections identified by LB or TB investigations was a newly detected infection. This case detection benefit will diminish in tandem with the decreasing future number of HCV LB and TB investigations involving transfusions before donor second generation anti-HCV donor screening. The current process of identifying and reporting suspect TT-HCV infections incurs delays in initiating follow-up LB or TB investigations.

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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

○移植後急性大脳辺縁系脳炎:臨床特性およびHHV6との関連性
背景:治療関連免疫抑制時においてヒトヘルペスウイルス6(HHV6)に起因すると考えられる急性大脳辺縁系脳炎が報告されている。しかし、この症候の臨床特性及び臨床検査特性はよく分かっていない。
方法:同種造血幹細胞移植(HSCT)後に急性大脳辺縁系脳炎を発症した患者9名の臨床特性、EEG、MRI、ならびに臨床検査特性を報告する。HHV6と本症候との関係性を調べるため、2003年3月17日～2005年3月31日までに当センターの診察を受けたHSCT患者全員から入手することのできたCSFのPCRによるHHV6検査結果を調べた。
結果:患者は、順行性健忘、不適切な抗利尿ホルモン分泌症候群、軽度CSF細胞増加症、一時的なEEG異常(顕性、不顕性けいれん発作を反映する場合が多い)を特徴とする、一定的で特徴的な臨床症候を示した。MRIでは、T2、FLAIR、DWI画像にて、鉤、扁桃体、内側嗅領、海馬領域内に高信号域を認めた。PCRを用いた初回腰椎穿刺CSFの検査では9名中6名がHHV6陽性であった。患者はいずれもfoscarnetまたはganciclovirによる治療を受けた。長期生存者の認知機能回復には、ばらつきがあった。1名の脳剖検では、大脳辺縁系グリオーシス及び扁桃体と海馬における著しい神経細胞脱落が示された。HHV6 CSF検査を2年間行ったHSCT患者27名のうち、臨床的大脳辺縁系脳炎を発症した患者にのみ陽性結果を認めた。
結論:同種造血幹細胞移植を行う患者には、神経症候の特徴的な移植後急性大脳辺縁系脳炎(PALE)のリスクがある。治療の検討には、積極的な発作管理と(場合によっては)抗ウイルス療法を含めるべきである。PALEは、CSF中のヒトヘルペスウイルス-6と関連付けられる可能性があるが、当該ウイルスの病原的役割についてはさらに検討が必要である。

報告企業の意見	今後の対応
同種造血幹細胞移植後に急性大脳辺縁系脳炎を発症した患者9名中6名が初回腰椎穿刺CSF検査でHHV6陽性となり、急性大脳辺縁系脳炎がCSF中のHHV6と関連付けられる可能性が示されたとの報告である。	今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

Post-transplant acute limbic encephalitis

Clinical features and relationship to HHV6

W.W. Seeley, MD*
F.M. Marty, MD*
T.M. Holmes, MD
K. Upchurch, MD
R.J. Soiffer, MD
J.H. Antin, MD
L.R. Baden, MD
E.B. Bromfield, MD

CME

Address correspondence and reprint requests to Dr. William W. Seeley, UCSF Memory and Aging Center, Box 1207, San Francisco, CA 94143-1207
wseeley@memory.ucsf.edu

ABSTRACT Background: Acute limbic encephalitis has been reported in the setting of treatment-related immunosuppression and attributed to human herpesvirus-6 (HHV6) infection. Clinical and laboratory features of the syndrome, however, have not been well characterized. **Methods:** We describe the clinical, EEG, MRI, and laboratory features of nine patients with acute limbic encephalitis after allogeneic hematopoietic stem cell transplantation (HSCT). To explore the relationship between HHV6 and this syndrome, we reviewed available CSF HHV6 PCR results from all HSCT patients seen at our center from March 17, 2003, through March 31, 2005. **Results:** Patients displayed a consistent and distinctive clinical syndrome featuring anterograde amnesia, the syndrome of inappropriate antidiuretic hormone secretion, mild CSF pleocytosis, and temporal EEG abnormalities, often reflecting clinical or subclinical seizures. MRI showed hyperintensities within the uncus, amygdala, entorhinal area, and hippocampus on T2, fluid-attenuated inversion recovery (FLAIR), and diffusion-weighted imaging (DWI) sequences. CSF PCR assays for HHV6 were positive in six of nine patients on initial lumbar puncture. All patients were treated with foscarnet or ganciclovir. Cognitive recovery varied among long-term survivors. The one brain autopsy showed limbic gliosis and profound neuronal loss in amygdala and hippocampus. Among 27 HSCT patients with CSF tested for HHV6 over a 2-year period, positive results occurred only in patients with clinical limbic encephalitis. **Conclusions:** Patients undergoing allogeneic hematopoietic stem cell transplantation are at risk for post-transplant acute limbic encephalitis (PALE), a distinct neurologic syndrome. Treatment considerations should include aggressive seizure control and, possibly, antiviral therapy. PALE can be associated with the CSF presence of human herpesvirus-6, but the pathogenic role of the virus requires further exploration.
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Human herpesvirus-6 (HHV6) is an emerging and controversial pathogen in neurology. In adults, the virus has been associated with a broad range of common disorders, including multiple sclerosis,[1,2] temporal lobe epilepsy,[3,4] and encephalitis,[5,6] especially in immunocompromised hosts.[7] HHV6 infects nearly all children by 3 years of age,[8] and two variants, A and B, have been identified. Primary HHV6-B infection can be asymptomatic or cause exanthem subitum[9] but most often presents acutely with fever, fussiness, rash, diarrhea, or seizures.[10,11] Both HHV6 variants are neurotropic in vivo and reside latently throughout the adult brain,[12] complicating any attempt to link HHV6 to cerebral disease.

Encephalitis following allogeneic hematopoietic stem cell transplantation (HSCT) was first attributed to HHV6 in 1994.[13] The virus has since been held responsible for numerous cases, but the literature consists mainly of isolated reports spanning diverse clinical syndromes from cranial neuropathies to coma.[14,15] In an intriguing minority of patients, the limbic system has been the primary or exclusive target. To date, nine adult limbic-predominant cases have been reported in isolation,[15-22] along with a pediatric series of five patients[23] and four patients identified as part of a longitudinal study of plasma HHV6 levels after HSCT.[24] This limbic subgroup is of biologic interest, because it is unclear how HHV6 might induce such targeted limbic inflammation in some patients but a more diffuse or multifocal encephalitis in others.

Supplemental data at www.neurology.org

*W.W.S. and F.M.M. contributed equally to this work.

From the Department of Neurology (W.W.S., T.M.H., K.U., E.B.B.), the Division of Infectious Diseases (F.M.M., L.R.B.), and the Division of Hematology/Oncology (R.J.S., J.H.A.), Brigham & Women's Hospital; Harvard Medical School (W.W.S., F.M.M., T.M.H., K.U., R.J.S., J.H.A., L.R.B., E.B.B.); and Dana-Farber Cancer Institute (F.M.M., R.J.S., J.H.A., L.R.B.), Boston, MA. W.W.S. is currently with the Department of Neurology, University of California, San Francisco. K.U. is currently with the Department of Neurosurgery, University of California, Los Angeles.

Disclosure: The authors report no conflicts of interest.

Table 1 Baseline patient characteristics

Patient	Age, y	Sex	Hematologic malignancy	Day 0*	Conditioning regimen	Donor relationship†	Stem cell source	GVHD prophylaxis	Engraftment	Acute GVHD	GVHD treatment
1	56	M	MDS	10/2001	Myeloablative cyclophosphamide and TBI	MRD	PBSC	CD8 T-cell depletion, tacrolimus	Day 12	Day 33, Grade III, GI, liver	Steroids, cyclosporine, tacrolimus, mycophenolate
2	53	M	CLL	11/2001	Non-myeloablative fludarabine and busulfan	MUD	PBSC	Prednisone, cyclosporine, mycophenolate	No neutropenia	Day 25, Grade IV, GI, liver, skin	Steroids, cyclosporine, tacrolimus, mycophenolate, daclizumab, infliximab
3	60	M	CML	1/2002	Non-myeloablative fludarabine and busulfan	MUD	PBSC	Prednisone, cyclosporine, mycophenolate	Day 7	Day 91, Grade II, skin	Steroids, mycophenolate, sirolimus, daclizumab
4	36	M	CML	3/2002	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus	Day 20	Day 15, Grade IV, skin, GI	Steroids, tacrolimus, sirolimus, mycophenolate, denileukin diftitox
5	32	M	HD	12/2003	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus, mycophenolate	Day 14	Day 85, Grade IV, liver	Steroids, tacrolimus
6	41	M	CLL	2/2004	Myeloablative cyclophosphamide and TBI	MUD	PBSC	Tacrolimus, sirolimus	Day 14	Day 13, Grade I, skin	Steroids, tacrolimus, sirolimus
7	59	M	CLL	10/2004	Non-myeloablative antithymocyte globulin fludarabine, melphalan	MUD	CORD	Cyclosporine, mycophenolate	Day 22	Day 22, Grade II, skin	Steroids, cyclosporine, mycophenolate
8	22	M	AML	1/2005	Myeloablative cyclophosphamide and TBI	MMUD	PBSC	Tacrolimus, sirolimus, methotrexate	Day 12	Day 13, Grade III, GI, liver	Steroids, tacrolimus, sirolimus, mycophenolate
9	38	M	AML	2/2005	Myeloablative cyclophosphamide and TBI	MMUD	PBSC	Tacrolimus, sirolimus, methotrexate	Day 12	Day 56, Grade IV, liver, GI	Steroids, mycophenolate

*Exact date omitted to protect patient anonymity.
†Patients were considered HLA-matched if all 6/6 HLA-A, -B, and -DR were identical.
GVHD = graft vs host disease; MDS = myelodysplastic syndrome; TBI = total body irradiation; MRD = matched-related donor; PBSC = adult peripheral blood stem cells; GI = gastrointestinal; CLL = chronic lymphocytic leukemia; MUD = matched-unrelated donor; CML = chronic myelogenious leukemia; HD = Hodgkin's disease; CORD = umbilical cord blood stem cells; AML = acute myelogenous leukemia; MMUD = mismatched-unrelated donor.

Here we describe nine patients with acute limbic encephalitis after HSCT, including six with PCR evidence of HHV6 DNA in the CSF. Although a subset of these patients has been described from a radiologic perspective,[25] this is the first adult series of post-transplant acute limbic encephalitis (PALE) based on clinical features. By characterizing this emerging syndrome, we seek to improve recognition of the illness and promote tractable studies of its pathogenesis.

METHODS **Patients.** Nine patients with acute limbic encephalitis were seen on the Brigham & Women's Hospital/Dana-Farber Cancer Care Hematopoietic Stem Cell Transplant Service between October 2001 and March 2005. Neurologic consultations, performed for each patient by one of the authors (W.W.S., K.U., T.M.H., or E.B.B.), were requested for unexplained amnesia, confusion, or a first-ever seizure. MR images were obtained from a Signa 1.5 T unit (GE Systems, Milwaukee, WI) using T1-weighted (before and after IV contrast), T2-weighted, fluid attenuation inversion recovery (FLAIR), diffusion-weighted (DWI), and apparent diffusion coefficient (ADC) sequences, though not all sequences were available for all patients. CSF assays for HHV6 DNA were performed at different reference laboratories during the study period. Samples from Patients 1 through 6 underwent non-quantitative PCR amplification with primers common to both HHV6 variants (A and B), precluding specification of the detected variant (Focus Technologies, Cypress, CA). For Patients 5 and 6, confirmatory testing was submitted for HHV6 DNA measurements using quantitative PCR (ViraCor Laboratories, Lee's Summit, MO).[26] Patients 6 through 9 had additional quantification of viral loads using a PCR assay with a quantitative range between 1,000 and 1,000,000 copies/mL that distinguishes between variants A and B (Associated Regional & University Pathologists [ARUP], Inc., Salt Lake City, UT).[27]

For all nine patients, relevant clinical data were collected, including age, sex, underlying hematologic malignancy, date of HSCT, conditioning regimen, source of stem cells, relationship and HLA matching of the donor and recipient, GVHD prophylaxis and treatment medications, and time and type of GVHD manifestations. Engraftment was defined as an absolute neutrophil count greater than 500 cells/μL for 2 consecutive days. GVHD was classified according to the consensus scale.[28] Information related to the clinical encephalitis was recorded, including day of onset after HSCT, presenting symptoms and findings on neurologic examination, EEG and MRI results, antiviral and anticonvulsive treatments used, concomitant medical and laboratory conditions, and clinical outcomes. Follow-up ended on June 30, 2005, for surviving patients.

Table 2 Post-transplant acute limbic encephalitis (PALE) after hematopoietic stem cell transplantation

Patient	PALE symptom onset*	Symptoms	1st EEG	1st MRI: foci of T2/FLAIR/DWI hyperintensity	Antiviral treatment	Anticonvulsive treatment	Concomitant conditions	Outcome
1	Day 25	Confusion, amnesia	Day 40: Diffuse theta slowing	Day 27: R>L uncus/amygdala, R=L hippocampus	Acyclovir ×10 d →valganciclovir, IVIg TIW	None	Hyponatremia (nadir 126 meq/L, Day 25)	Alive Day 1351; Good cognitive recovery
2	Day 29	Amnesia, episodic staring with oral automatisms	Day 75: PLEDS, L anterior temporal	Day 31: R>L uncus/amygdala, hippocampus	Acyclovir→ganciclovir × 12 d →foscarnet × 18 d	Phenytoin→ levetiracetam	Hyponatremia (nadir 130 meq/L, Day 35)	Died Day 144 from severe GI GVHD and bacteremia; poor cognitive recovery; no autopsy
3	Day 55	Amnesia, episodic confusion with complex motor behavior	Day 60: Bitemporal slowing with sharp waves	Day 60: R>L uncus, R=L hippocampus	Acyclovir→valganciclovir × 21 d	Valproate & levetiracetam	Hyponatremia (nadir 118 meq/L, Day 60); posterior leukoencephalopathy (cyclosporine)	Alive Day 1254; poor cognitive recovery with persistent episodic memory impairment
4	Day 61	Confusion, amnesia, visual hallucinations	Day 62: Excessive beta activity, diffuse theta activity	Day 62: R=L uncus, hippocampus	Valganciclovir × 10 d	Gabapentin (ongoing for neuropathy)	Hyponatremia (nadir 129 meq/L, Day 68); *S aureus* bacteremia, DAH	Died Day 157 from refractory GI GVHD and enterococcal sepsis; good cognitive recovery; autopsy (see text)
5	Day 31	Generalized tonic-clonic seizures, confusion, amnesia, emotional outbursts	Day 31: Diffuse theta and delta slowing, nonspecific sharps	Day 31: R>L uncus/amygdala, hippocampus	Acyclovir × 1 d→ganciclovir × 12 d →foscarnet × 31 d→repeat foscarnet × 11 d	Phenytoin→ levetiracetam	Hyponatremia (nadir 128 meq/L, Day 34); VOD of the liver, ARF	Died Day 108 from liver GVHD, VOD, ARF; modest cognitive recovery; autopsy excluded brain
6	Day 25	Generalized tonic-clonic seizures, confusion, amnesia, emotional outbursts (aggressive, hypersexual)	Day 27: L anterior temporal spikes, intermittent runs of L>R temporal theta; R frontotemporal PLEDS 4 d later	Day 26: L>R uncus, hippocampus	Acyclovir × 1 d →foscarnet × 28 d; IVIg	Phenytoin→ levetiracetam	Hyponatremia (nadir 126 meq/L, Day 31)	Alive Day 663; modest cognitive recovery, persistent episodic memory impairment
7	Day 32	Confusion, amnesia, anomia; depression and withdrawal after antiepileptic treatment	Day 35: R frontotemporal PLEDS	Day 32: normal; Day 37: R=L amygdala, hippocampus	Acyclovir→ganciclovir × 5 d→ foscarnet × 28 d	Levetiracetam	Neurocardiogenic syncope; no hyponatremia, (nadir 135 meq/L, Day 41); hypernatremia to 155, peak Day 66)	Alive Day 251; modest cognitive recovery, persistent episodic memory impairment
8	Day 14	Agitation and aggression followed by lethargy and hypercarbic respiratory failure; after extubation on Day 22, amnesia and confusion	Day 26: R>L theta and delta activity; periodic right temporal sharp waves	MRI not done due to indwelling bullet and TEN; Day 23: CT normal	Foscarnet × 4 d→ganciclovir × 6 d→foscarnet × 8 d	Levetiracetam	Hyponatremia (nadir 130 meq/L, Day 15); aspiration pneumonia, TEN vs skin GVHD	Died Day 42 from MRD-*Acinetobacter baumannii* bacteremia and septic shock, persistent TEN; recovery not assessable; no autopsy
9	Day 18	Confusion, fever, resolving to amnesia; one emotional (hypersexual) outburst	Day 28: Poor organization, theta and delta slowing; Day 68: Diffuse theta and delta activity, one run of asymmetric beta followed by sharp alpha and 4–5 Hz R temporal theta	Day 18: R=L uncus/amygdala, hippocampus	Foscarnet × 28 d	Levetiracetam	Hyponatremia (nadir 126 meq/L, Day 18); incident VOD, ARF, and aspiration pneumonia	Died Day 114 from persistent liver GVHD and VOD, ARF; modest cognitive recovery; no autopsy

*Days refer to interval from hematopoietic stem cell transplantation.
IVIg = IV immune globulins; TIW = three times weekly; PLEDS = periodic lateralized epileptiform discharges; GI = gastrointestinal; GVHD = graft vs host disease; DAH = diffuse alveolar hemorrhage; VOD = veno-occlusive disease; ARF = acute renal failure; TEN = toxic epidermal necrolysis; MRD = multi-drug resistant.

Clinical database search. The first PALE cases to be recognized at our center were encountered in 2001. Thereafter, HHV6 PCR testing was routinely included when HSCT patients underwent lumbar puncture for an acute neurologic change. To evaluate the specificity of a positive HHV6 CSF PCR assay for PALE and to minimize the possibility of inception bias, we searched the Partners Healthcare System Research Patient Data Repository for all CSF HHV6 PCR determinations performed for HSCT recipients, beginning when the test became available to all clinicians in March 2003 and ending on March 31, 2005. Medical records from all tested patients were examined for clinical and radiographic (MRI) evidence of limbic encephalitis. Indications for CSF sampling and final diagnoses rendered for each indication were recorded. When testing limbic encephalitis patients and patients identified through the database search,

the reference laboratories were unaware of the clinical syndromes that prompted HHV6 testing.[26,27]

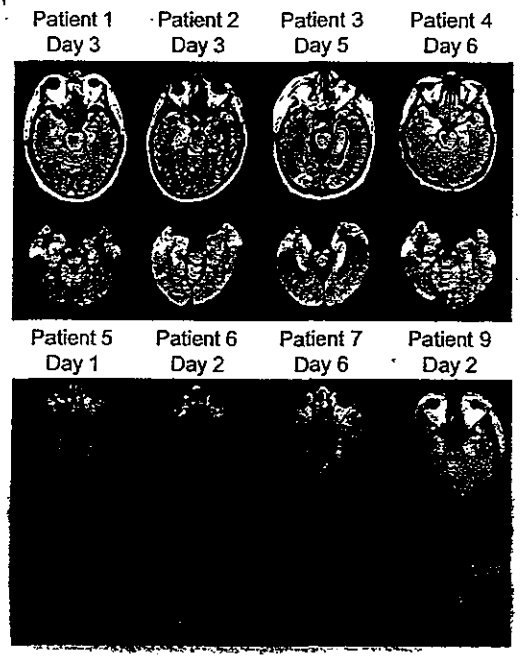
The study was approved by the institutional review boards for the Partners Healthcare System and the University of California, San Francisco.

RESULTS **Clinical features.** We encountered nine HSCT patients with an acute limbic encephalitis syndrome between October 2001 and March 2005. During this period, 584 patients underwent allogeneic HSCT, suggesting a cumulative PALE incidence of 1.5%. Table 1 summarizes the baseline clinical characteristics of the patients in the series. All were men with a median age of 41 years (range 22 to 60 years) who underwent HSCT for a range of hematologic malignancies (table 1). Donors were unrelated to the recipient for all but Patient 1, who underwent a T-cell depleted transplant from a HLA-matched sibling donor. Six patients underwent myeloablative conditioning, three nonmyeloablative. All developed GVHD. Severity and organ involvement varied. GVHD diagnosis and treatment preceded onset of limbic encephalitis in five and followed it in four patients. Prophylactic and treatment GVHD immunosuppressive regimens also varied (table 1). Engraftment preceded neurologic symptom onset in all patients.

Table 2 summarizes the clinical characteristics of the limbic encephalitis syndrome. Neurologic symptoms began a median of 29 days after HSCT (range 14 to 61 days). Herpes simplex and herpes zoster virus prophylaxis with oral or IV acyclovir was ongoing in all patients, and cytomegalovirus (CMV) surveillance was performed one to two times per week by use of a whole-blood CMV hybrid capture assay. Clinical features common to all patients included acute onset over 1 to 3 days, dense anterograde amnesia, and patchy retrograde amnesia for up to 4 years. All patients lost memory of their underlying HSCT-requiring condition. Patient 1 failed to recognize an examiner returning from a 3-minute telephone call. Patient 3 was surprised to find a tunneled central venous catheter in his upper chest wall when prompted by his wife to clean it. Patient 4 had no memory of the September 11, 2001, tragedy when queried in April 2002. Language, praxis, and visuoconstructive skills were spared on routine bedside testing. A confusional state was present at symptom onset in Patients 1 and 4 through 9 but gave way to amnesia within the first several days. Fever was present only in Patients 5 and 6 and could be attributed to ongoing GVHD or concomitant infection. Clinical seizures occurred without question in Patients 2, 3, 5, and 6, were suspected in Patients 7 through 9, and typically did not respond to the first medication given (table 2). Patients 2 and 3 had complex partial seizures that were ultimately controlled with levetiracetam, valproic acid, or both. In Patients 5 and 6, a generalized tonic-clonic seizure marked symptom onset. Periods of intense agitation followed antiepileptic treatment in both cases. Patient 6 cursed at family members (including a small child) and struck a nurse. Patient 7 was started on levetiracetam after his EEG and became emotionally withdrawn for several days thereafter. Hyponatremia due to inappropriate production of antidiuretic hormone (SIADH) heralded or began with symptom onset in all but Patient 7. No other consistent general laboratory abnormality was seen.

Clinical course varied. In most instances, suspicion for HSV encephalitis prompted acyclovir treatment within 1 to 2 days of symptom onset. Therapy changed when HSV PCR testing was negative or concern for HHV6 rose (table 2). Cognitive recovery ranged from excellent to none. Upon retesting, anterograde amnesia had improved considerably in Patients 1 and 4, little in Patient 3, and modestly in Patients 6, 7, and 9. Patient 2 remained densely amnesic prior to his death from GVHD and sepsis. Patient 5 made modest gains in anterograde memory before developing GVHD, veno-occlusive disease of the liver, and ultimately death from pneumonia. Patient 6 was examined in the neurology clinic 15 months after his illness. Digit span backward was six. On a verbal learning test, he was able to recall 7/7 words after the first entrainment trial. After a 60-second distraction, he recalled 5/7, at 3 minutes 5/7, and at 10 minutes he was able to recall only 3/7

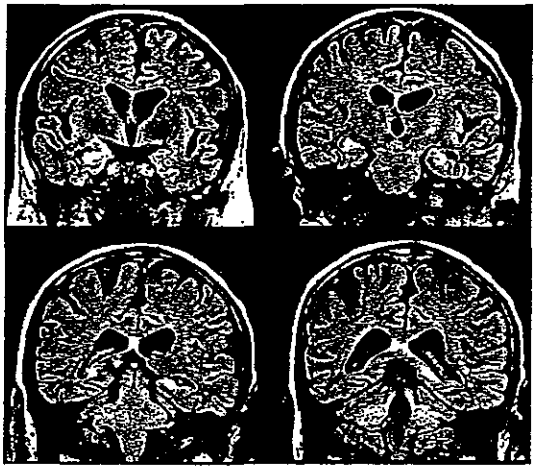
Figure 1 Initial MRI findings, with days from symptom onset indicated



Axial fluid attenuation inversion recovery (FLAIR) (top) and diffusion-weighted (bottom) sequences demonstrate limbic system involvement, including the uncus, amygdala, and anterior hippocampus in all patients. For Patient 6, only coronal FLAIR images from day 2 were available. An axial FLAIR image from day 6, with findings similar to those on day 2, is shown. The left side of each image corresponds to the right side of the brain

Coronal FLAIR MR images from Patient 1 demonstrate the focality of the encephalitis, which can be traced bilaterally from the uncus through the hippocampal body and into the fornix. The amygdala is partly affected and the parahippocampal gyrus is conspicuously spared. The left side of each image corresponds to the right side of the brain.

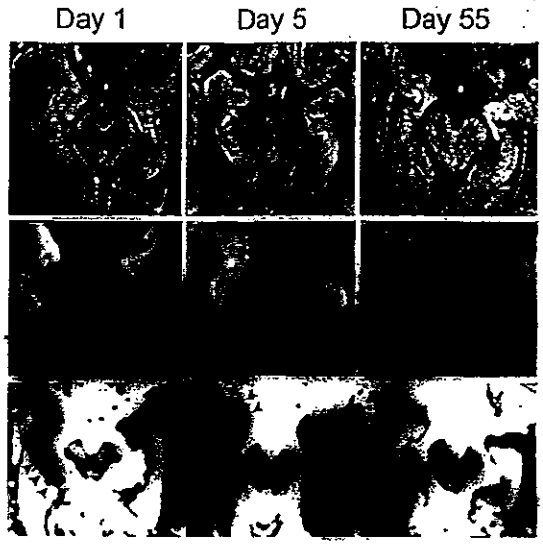
Figure 2 Coronal fluid attenuation inversion recovery (FLAIR) MR images from Patient 1



words. He requires a global positioning system device to drive without getting lost. Patient 7 performed similarly when examined 4 months after his illness during an unrelated hospital admission. Patient 8 developed toxic epidermal necrolysis due either to trimethoprim-sulfamethoxazole or severe GVHD. He required intubation, mechanical ventilation, and heavy sedation that precluded cognitive follow-up. He later died from septic shock caused by multidrug-resistant *Acinetobacter baumannii*. Absent or early treated seizures were associated with a better cognitive outcome. Patients 2 and 3 recovered least and both had refractory complex partial or clinically "silent" electrographic seizures early in their course.

MRI findings. All but Patient 8 underwent MRI of the brain. Without exception, focal medial temporal abnormalities were identified (table 1, figure 1). This radiographic signature involved circumscribed, bilateral, non-enhancing, T2/FLAIR/DWI hyperintensities within the uncus, amygdala, and hippocampal body, typically extending to the entorhinal cortex and subiculum. Parahippocampal gyrus was conspicuously spared. The posterior extent of the lesions varied but reached as far as the intersecting crura of the fornix (figure 2). Some asymmetry was common. Initial MR imaging in Patient 3 also revealed a posterior leukoencephalopathy that resolved after discontinuation of cyclosporine. ADC maps were available for four patients and suggested true restricted diffusion in Patient 5 (figure 3) but T2 "shine through" effects in the others (Patients 6, 7, and 9).[25] Serial imaging of Patients 1, 5 through 7, and 9 revealed a consistent radiographic progression (figure 3). Within affected areas, increased DWI signal appeared acutely, but over time increasing T2/FLAIR signal emerged as diffusion characteristics returned to normal. Four patients were rescanned at least 6 weeks from symptom onset. In each case, hippocampal volume was notably reduced (see figure 3); whether this finding represents atrophic change or resolution of edema is uncertain since patients were not imaged prior to developing limbic encephalitis.

Figure 3 MR signal changes over time in Patient 5



Limbic fluid attenuation inversion recovery hyperintensities (top row) were prominent in the early subacute phase, even as diffusion-weighted imaging (DWI) signal (middle row) waned. Apparent diffusion coefficient maps (bottom row) showed restricted diffusion within DWI-bright regions (arrowheads). Prominent hippocampal atrophy was demonstrated 55 days from symptom onset (right column). The left side of each image corresponds to the right side of the brain.

EEG findings. EEG was performed for all patients due to observed or suspected seizures. Focal abnormalities were seen over the temporal or frontotemporal leads in seven of nine patients, usually involving both sides. All seven had epileptiform activity including two with electrographic seizures, three with periodic lateralized epileptiform discharges (PLEDS), one with both seizures and PLEDS, and one with sporadic interictal discharges. The remaining two patients had diffuse slowing, in one case recorded on the same day as a convulsive seizure. A representative EEG tracing from Patient 6 illustrates the epileptogenic nature of PALE (figure E-1 on the *Neurology* Web site at www.neurology.org).

CSF and HHV6 PCR results. Findings at lumbar puncture are shown in table 3. No specimen was xanthochromic. Mild CSF pleocytosis with lymphocyte predominance was present in all but Patient 4 (median 5 leukocytes/mm^3, range 1 to 41). Protein elevations were typically mild (median 48 mg/dL, range 19 to 189). Patients 1, 2, and 5 through 9 tested positive for HHV6 by PCR on at least one CSF sample. Traumatic blood contaminated the HHV6-positive specimen from Patient 2, raising the possibility that HHV6 DNA from blood was detected. Further, this specimen was obtained 3

Table 3 CSF analysis and human herpesvirus-6 (HHV6) PCR results

Patient	PALE onset	LP timing	Protein mg/dL	Glucose mg/dL	WBC cells/mm^3 (% lymphs)	RBC cells/mm^3	CSF HHV6 PCR copies/mL	HHV6 variant
1	Day 25	Day 26	45	72	4 (93)	0	Positive	ND
		Day 40	58	80	2 (82)	2	Negative	
		Day 158	91	46	14 (90)	0	Negative	
2	Day 29	Day 30	97	56	12 (93)	0	Negative	
		Day 51	189	71	41 (98)	178	Negative	
		Day 125	19	78	1 (88)	3,444*	Positive	ND
3	Day 55	Day 56	53	69	5 (91)	0	Negative	
		Day 58	33	73	7 (82)	3	Negative	
		Day 71	90	62	7 (92)	0	Negative	
4	Day 61	Day 63	29	119	1 (66)	0	Negative	
5	Day 31	Day 32	106	76	25 (5)	47,000*	Positive	ND
		Day 81	37	75	17 (82)	4	Positive: 100	B
		Day 102	32	84	2 (96)	1	Negative	
6	Day 25	Day 25	64	74	7 (64)	138	Positive	ND
		Day 37	44	59	7 (85)	3	Negative	
7	Day 32	Day 33	48	71	16 (92)	30*	Positive: >999,000†	B
		Day 45	50	64	7 (88)	0	Positive: <1,000‡	B
		Day 66	81	62	7 (90)	23	ND	
8	Day 14	Day 25	28	59	3 (58) 27% monocytes	18	Positive: <1000§	B
9	Day 18	Day 19	42	78	2 (44)	0	Positive: 203,000‖	B
		Day 34	49	112	3 (92)	44	Positive: <1,000	B
		Day 46	42	148	1 (91)	2	Negative	

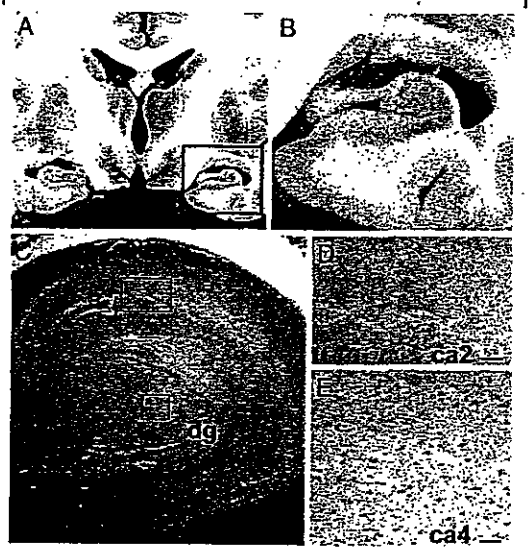
*Specimen obtained through a traumatic lumbar puncture yielding pink CSF.
†Plasma sample on Day 36 showed a HHV6 viral load of 186,000 copies/mL.
‡Plasma sample on Day 45 showed a HHV6 viral load of <1,000 copies/mL.
§Plasma sample on Day 17 showed a HHV6 viral load >999,999 copies/mL. Foscarnet was begun, and a follow-up viral load was 1,430 copies/mL (Day 30).
‖Plasma sample on Day 22 showed a HHV6 viral load of 4,070 copies/mL.
¶Plasma sample on Day 33 did not reveal HHV6 viremia.
PALE = post-transplant acute limbic encephalitis; LP = lumbar puncture.

months after symptom onset, making it unlikely the culprit pathogen in the limbic encephalitis. Initial CSF samples from Patients 5 and 7 were also blood-tinged, but their second samples were untainted and still positive. Other microbiologic studies performed on CSF varied due to clinician preference, season, and CSF availability, but all patients were negative for HSV 1 and 2, CMV, EBV, JCV, and VZV by PCR. Bacterial and fungal cultures, stains for acid fast bacilli, and VDRL were unrevealing. Patients tested for paraneoplastic autoantibodies (anti-Hu, anti-Ma/Ta, anti-CV2) in the CSF (Patients 1 and 3) or serum (Patient 6, anti-Hu and anti-Ma/Ta only) were found negative.

Neuropathology. Brain autopsy was performed only on Patient 4, who died 3 months after his encephalitic episode due to unrelated causes (table 2). Microscopic examination (figure 4) revealed severe neuronal loss and gliosis in CA1 and CA4 regions of the hippocampus and the amygdala, with milder gliosis of CA2, CA3, subiculum, fornices, insula, and cingulate. The hippocampus showed no immunopositivity for HSV, CMV, or VZV antigens. A single microglial nodule was seen in the fornix. No other focal abnormalities were identified. HHV6 immunohistochemistry was not performed.

Clinical database findings: CSF HHV6 PCR results over a 2-year period. Between March 17, 2003, and March 31, 2005, CSF aliquots from 41 lumbar punctures performed on 27 post-HSCT patients were submitted for HHV6 detection, corresponding to 6.4% of patients transplanted during this period. Twelve samples corresponded to Patients 5 through 9 of the present series. HHV6 DNA was detected in none of the 29 samples from the remaining 22 patients. From these patients, initial specimens were obtained a median of 51 days after HSCT (range, 16 to 733 days). Indications for CSF sampling were an

Figure 4 Postmortem findings in Patient 4, 3 months after clinical encephalitis



Gross findings (A, B) included atrophy of the hippocampus, right worse than left, and amygdala (not shown). Microscopic examination of the hippocampus (C–E) revealed profound neuronal loss in CA1 and CA4, accompanied by astrocytic gliosis (E). CA2 and 3 were also injured, though less severely (C, D). Hematoxylin & eosin stain. Scale bars correspond to 100 μm (C), 50 μm (D), and 20 μm (E). Regions boxed in A and C are magnified in B, D, and E. The left side of each image corresponds the left side of the brain. Dg = dentate gyrus.

acute confusional state in nine, decreased sensorium in seven, seizures in three, and transverse myelitis, polyradiculitis, and a catatonic state in one each. Final diagnoses were encephalopathy due to sepsis, renal, or hepatic failure in nine, tacrolimus-associated reversible posterior leukoencephalopathy in three, and a case each of leukemic relapse, encephalomyelitis, focal occipital encephalitis vs glioma, Lyme neuroborreliosis,[29] oversedation, subarachnoid hemorrhage, seizure disorder, staphylococcal meningoencephalitis, herpes zoster myelitis, and depression. No additional cases of limbic encephalitis were noted. Therefore, during this 2-year period, CSF HHV6 PCR was positive only in the context of acute limbic encephalitis.

DISCUSSION Based on this series and prior reports,[15-21,23-25] we propose that patients undergoing allogeneic HSCT are at risk for post-transplant acute limbic encephalitis, or PALE, a distinct neurologic syndrome. Children and adults are susceptible. Men appear more vulnerable, as we have yet to observe the illness in women though they constitute 40 to 45% of our annual HSCT cohorts and 10 of 13 PALE patients reported in the literature have been men.[15-22,24] Consistent PALE clinical features include marked anterograde amnesia, seizures or temporal lobe EEG abnormalities, SIADH, and mild CSF pleocytosis. The syndrome is accompanied by an MR signature of bilateral, nonenhancing, medial temporal lobe T2/FLAIR/DWI hyperintensities sharply demarcated by parahippocampal gyrus sparing. PALE is associated with a short interval from transplantation (15 to 60 days) and may be more common after unrelated donor HSCT and myeloablative conditioning. Due to reports of a nearly identical illness after solid organ transplantation[30,31] and the likelihood of a shared pathophysiology, we offer the term PALE for its unifying and descriptive qualities.

The natural history of PALE has not been previously described. None of the patients in the present series died as a direct result of encephalitis (table 2). Patients alive 6 months after HSCT (Patients 1, 3, 6, and 7) had persistent mild to moderate neuropsychological deficits largely confined to episodic memory. Factors influencing outcome remain uncertain, but early seizure control may significantly improve prognosis.

What is the pathophysiology of PALE? In this series, symptoms arose early in the post-HSCT course (<61 days) but always after engraftment. CSF profiles suggested increased blood–brain barrier permeability and mild pleocytosis, both attributable to low-grade inflammation, seizures, or both. Minor subarachnoid hemorrhage with xanthochromia, as seen with the necrotizing inflammation of HSV encephalitis, was absent. EEG often revealed the epileptogenic nature of the illness and supported the clinical impression of interictal as well as ictal temporal dysfunction. Serial MRI characteristics of PALE highlight its urgency. ADC maps at times showed restricted water diffusion in affected tissues, suggesting that acute DWI hyperintensities seen in all patients were not only due to "shine through" T2 prolongation effects from vasogenic edema. Known pathologic-biophysical correlates of reduced ADC include ischemic cytotoxic edema (with failure of energy dependent ion homeostasis), seizure-related excitotoxicity/hyperperfusion (with glucose utilization exceeding oxygen delivery, leading to cellular lactate accumulation and osmotic shifting of extra- to intracellular water), and acute myelin vacuolization.[32,33] Although our patients were not clinically seizing at the time of imaging, subclinical epileptiform activity was often found when sought. Considering the prior report of PET hypermetabolism in children with PALE,[23] our DWI/ADC findings suggest a reversible metabolic supply/demand mismatch during the acute epileptogenic phase. Rising T2 and falling DWI signals of the early subacute phase suggest that vasogenic edema may linger even after seizures are controlled. Apparent hippocampal atrophy following PALE suggests that the excitotoxicity of the illness, though treatable, can lead to irreversible limbic injury.

The pathophysiology of PALE is compatible with an infectious or inflammatory etiology. This study provides modest support for the alleged role of HHV6 in the syndrome, with the caveat that dif-

ferent HHV6 PCR assays were employed during the study period as clinical practice evolved. Nonetheless, six of nine cases (Patients 1, 5 through 9) had a positive initial CSF HHV6 PCR assay. When available, quantitative HHV6 PCR showed rapid treatment-related reductions in HHV6 viral load not always accompanied by clinical improvement. CSF HHV6 PCR from Patients 2 through 4 was negative or indeterminate. Therefore, despite the clinical consistency of this series, CSF HHV6 DNA was detected in only two-thirds of the patients. This incomplete association of HHV6 with PALE should trigger several important questions about this clinical syndrome.

Is PALE caused by HHV6 and, if so, what are the sensitivity and specificity of CSF PCR assays? PALE is a newly described, infrequent, and underrecognized syndrome, making the test characteristics of HHV6 CSF PCR difficult to evaluate, even at centers that are attuned to the diagnosis. In the only prior study to explore the significance of HHV6 PCR in immunocompromised patients, HHV6 was found in CSF from 1/107 asymptomatic control patients who underwent standard non-HSCT treatment for leukemia, 0/11 post-HSCT patients with known causes of their "CNS symptoms," and 5 of 11 post-HSCT patients with symptoms for which no cause was found.[34] None of the 5 HHV6 PCR-positive cases had a syndrome typical of PALE. During a 2-year interval at our institution, quantitative CSF PCR assays for HHV6[27] were positive in all patients (5/5) with a clinical diagnosis of PALE and negative among 22 HSCT patients who lacked core features of PALE but were tested in the context of other acute neurologic syndromes.

How often does PALE arise without HHV6? To our knowledge, Patients 2 through 4 are the only HHV6-negative PALE cases on record. Each developed PALE between 2001 and 2002 when a nonquantitative, less sensitive assay was in use at our institution. Nonetheless, their results suggest that HHV6-PCR-negative PALE can occur and that scattered cases may have gone unreported at our center or elsewhere. Awareness of PALE is limited, and such cases could have been wrongly attributed to herpes simplex encephalitis, even in the face of a negative CSF HSV PCR assay. In a recent longitudinal study of three HSCT patients with encephalitis, high levels of HHV6 DNA and mRNA were detected in postmortem hippocampus and other limbic regions, even though CSF HHV6 DNA had fallen to undetectable levels in the weeks before death.[35] Further prospective studies are needed to more completely assess HHV6 PCR test characteristics in the context of PALE.

If PALE is caused by HHV6, what is the range of clinical phenomena that HHV6 can effect? Two reviews of post-transplant HHV6-associated encephalitis conducted in 2000[14,15] demonstrated heterogeneous syndromes and a paucity of neuroimaging abnormalities. Only three of the combined 14 patients described had clinical and imaging patterns characteristic of PALE. Therefore either HHV6 has protean manifestations or its detection can be nonspecific, perhaps reflecting an innocent bystander brought forth by an unidentified primary disease. PALE could reflect HHV6 reactivation, as the virus resides latently in at least 85% of non-neurologic autopsy control brains[12] and has a predilection for the medial temporal lobes.[4]

Limbic encephalitis (LE) has become an important and multifaceted syndrome in neurology. Apart from the rapidity with which it evolves, PALE bears a strong clinical resemblance to paraneoplastic LE, classically a subacute illness associated with occult small cell lung carcinoma and anti-Hu antibodies.[36] During the past decade, LE has been linked to multiple underlying malignancies and autoantibodies[37] and can occur without cancer, at times accompanied by autoantibodies to voltage-gated potassium channel isoforms expressed by hippocampal neurons.[38,39] The related construct of non-herpetic acute limbic encephalitis (NHALE) has emerged in the Japanese literature,[40] occurs in immunocompetent hosts after routine viral illness, causes hippocampal DWI hyperintensity, and can respond to immunosuppressive therapy.[41] In view of this gathering complexity, PALE provides a useful new model of LE by occurring in a context unfit for most forms of autoimmunity. Conversely, studies of autoimmune LE could reveal parainfectious immune mechanisms that prove relevant to PALE. Many LE variants focus their attack on limbic structures through mysterious means; whether HHV6 plays a role in all, some, or none of them remains an open question.

PALE is a clinically recognizable entity that requires a low threshold for diagnosis. Post-transplant patients with acute alterations in mental status, prominent amnesia, or unexplained seizures should undergo MRI with diffusion-weighted imaging, lumbar puncture, and CSF PCR testing for HHV6 and other potential culprit viruses. Seizures should be assumed ongoing or impending and treated early to prevent irreversible hippocampal injury. The pathogenesis of PALE deserves further investigation, and prospective antiviral treatment trials for PALE are needed. We hope this series will stimulate clinicians to report PALE cases whether or not an identifiable viral etiology is found.

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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

○献血者のHEVスクリーニング

背景:本邦の主要なHEV伝播ルートは、動物食肉由来の食物感染である。最近我々は、輸血により伝播したE型肝炎症例を報告した。この症例の原因献血者は、動物由来食物ルートより感染したと考えられ無症状であった。

目的:1)献血者のHEVスクリーニングにより輸血伝播HEV感染を予防すること。2)HEV感染の自然史を明らかにすること。

方法:HEV-RNAの検出と定量はTaqMan real-time RT-PCR法により実施し、HEV-RNAを in vitroで転写したcDNA テンプレートを定量のスタンダードとして用いた。HEV 遺伝子型は、ORF1配列を基にダイレクトシークエンス法および系統樹解析により判定した。遺伝子組み換えHEVウイルス様粒子や市販のキットを用いたin-house ELISAにより、抗HEV抗体を検出するELISA法を実施した。

結果:2005年1月～2006年8月に北海道において献血者のHEVスクリーニングを行った。388,119名のうち、男性33名(1/7,120)、女性22名(1/6,962)がHEV-RNA陽性で、genotype 3が優勢であった(G3/G4=51/4)。55名中40名は献血時のHEV抗体陰性であり、後に陽性となった。フォローアップ検査では、一部のHEV陽性献血者のALT値が67～333 IU/Lまで上昇したが自覚症状のある献血者はいなかった。HEV-RNAは最長37日間検出され、最短で献血の6日後に検出されなくなった。IgM抗体は最短51日で検出されるようになった(献血後237日以上存在した)。55人分の献血血液のうち7製剤が輸血された。2製剤は2名の患者にHEV感染を発症させ、別の2製剤は2名に輸血されたがHEVを発症させず、3製剤では患者が輸血後30日以内に死亡したため不明であった。献血者の感染ルートは明らかではないが、献血者の多くに過去60日以内の動物内臓肉摂食歴があり、そうした肉ではHEV汚染が時々報告されているため、動物由来食物感染による可能性が高い。

結論:献血者の7000人に1人の割合でHEV-RNAが検出された。HEVキャリアの一部にALT値上昇が認められたが、ほとんどが無症状でHEV抗体陰性であった。HEVウイルス血症は、献血後最長で37日間検出された。HEV陽性献血者由来の輸血を受けた患者7名のうち、少なくとも2名が感染した(1名は顕性、もう1名は不顕性)。献血者の感染ルートは不明だが、動物食肉由来の食物感染が示唆されている。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
北海道の献血者のHEVスクリーニングを行ったところ、献血者の7000人に1人の割合でHEV-RNAが検出され、HEV陽性献血者由来の輸血を受けた患者7名のうち、少なくとも2名が感染した。献血者の感染ルートは明らかではないが、動物由来食物感染が示唆されたとの報告である。	日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断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。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。また、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を輸血用から排除している。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。

(1)

Poster Session
6.2 Blood safety - transfusion transmitted disease (TTD) - hepatitis

P203

HEV SCREENING FOR BLOOD DONORS

Ikeda H, Sakata H, Matsubayashi K, Tokushima E, Tanaka S, Sato S, Kato, T
Hokkaido Red Cross Blood Center, Sapporo, Japan

Background: Zoonotic food-borne infection is a major transmission route of HEV in Japan. Recently, we reported a transfusion-transmitted hepatitis E case (Ikeda H et al., Transfusion 2005; 45: SP207), where the causative donor was apparently infected by zoonotic food-borne route and remained asymptomatic.
Aims: 1)To prevent transfusion-transmitted HEV infection by screening blood donors for HEV.
2) To clarify natural history of HEV infection.
Methods: HEV-RNA detection and quantitation was carried out by TaqMan real-time RT-PCR using in vitro-transcribed RNA from HEV cDNA template as quantitation standards. HEV genotype was determined by PCR direct sequencing and phylogenetic analysis base on ORF1 sequences.
ELISA for anti HEV was carried out with in-house ELISA using recombinant HEV virus-like particles(Li TC et al., J Med Virol 2000;62:327–33.) and/or commercial kit (Cosmic Corporation. Co., Ltd., Japan)
Results: Blood donors were screened for HEV-RNA from January 2005 to April 2006. Of 388, 119 donations, 33 males (1/7120) and 22 females (1/6962)were HEV-RNA(+). Genotype 3 was predominant(G3 /G4 = 51/4). Of 55 HEV-RNA(+)donors, 40 were seronegative at donation and eventually seroconverted. Follow-up studies revealed that no HEV(+)donor had subjective symptom, although ALT levels went up to 67–333 IU/L in some of them. HEV-RNAs were detected in 37 days at the longest or undetected in 6 days at the earliest after the donations. Their IgM antiHEV became detectable in 51 days at earliest or lasted more than 237 days after the donations.
Of 55 donations, seven were used for transfusion; two caused HEV infection in two patients; two did not cause infection in two patients; three were unknown because patients died before 30 days after transfusion. Although their infection routes were not very clear, zoonotic food-borne route was likely, because most of them had history of ingesting animal meat derived from internal organs in the past 60 days and such meat was reported to be sometimes contaminated with HEV (Kato M, et al., Kanzo 2004; 45:688).
Conclusion: HEV-RNA was detected in our blood donors at the rate of about 1/7000. HEV carriers were mostly asymptomatic and seronegative, although high ALT level was observed in some. HEV viremia was detected in 37 days at the longest after donation. Of seven patients who were transfused with HEV positive blood, at least two were infected (one symptomatic and the other asymptomatic). Although their infection route was not clear, zoonotic food borne infection was suggestive.

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研究報告の概要

○輸血後E型肝炎の2例
背景と目的:近年、本邦におけるHEVの感染経路や、その臨床像が明らかになりつつある。今回、発症前からのウイルス血症推移、肝炎発症と沈静化までの経過を観察しえた輸血後E型急性肝炎(AH-E)2例を経験したので報告する。
症例1:60歳代、男性。2003年9月NH Lymphomaと診断され、治療中、血小板減少に対し照射濃厚血小板(Ir-PC)を輸血。後日、輸血されたIr-PCがHEV陽性であることが判明し、同年10月AH-E発症の可能性が高いと考えられ、精査加療目的で当院へ転院となる。輸血21日目にHEV RNA(genotype 4)を検出、対数増殖期を経て44日目に7.2log copies/mlとピークを示した。ALTは21日目に正常上限を越え59日目に673IU/Lまで上昇した。Genotype 4感染による肝炎の重症化を懸念し、IFNを使用したがウイルス血症、肝炎は改善せず、PSL(プレドニゾロン)30mg内服によりALTは低下、その後抗HEV IgGの出現と同期し87日目にウイルス血症も改善した。
症例2:70歳代、男性。2005年8月、急性心筋梗塞治療中に投与されたIr-PCが後にHEV陽性と判明し、AH-E精査加療目的に当院へ転入となる。HEV RNA(genotype 3)は輸血後3日目に同定され、54日目に7.4log copies/mlとピークに達し、ALTは17日目に正常上限を越え65日目に972IU/Lまで上昇した。抗HEV IgM、IgGが60日目に陽性化後HEV、トランスアミナーゼは低下し、113日目にウイルス血症も改善した。
まとめ:HEVウイルス血症は10～22日の潜伏期間を経て発現、対数増殖後約50日前後にピークを示し、その直後にAST、ALT上昇と血中HEV抗体の出現を順次認めた。
結語:輸血後AH-E2例の全経過を追跡しえた。本例のように、発症前から血中ウイルス感染マーカーを生化学所見、経過と同時に観察したAH-E例は実験例を除くと未見であり、貴重な症例と考えられる。

使用上の注意記載状況・その他参考事項等

合成血「日赤」
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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
発症前からのウイルス血症の推移、肝炎発症から沈静化までの経過を観察しえた輸血後E型肝炎症例において、HEVウイルス血症は10～22日の潜伏期間を経て発現、対数増殖後約50日前後にピークを示し、その直後にAST、ALT上昇と血中HEV抗体の出現を認めたとの報告である。	日本赤十字社では、厚生労働科学研究「E型肝炎の感染経路・宿主域・遺伝的多様性・感染防止・診断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。北海道における輸血HEV感染報告を受け、試験的に北海道では研究的NATを行うなど安全対策を実施している。また、輸血による肝炎ウイルス感染防止のため、血液中のALT値61IU/L以上の血液を輸血用から排除している。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。

O-178　輸血後E型肝炎の2例

松居剛志[1], 姜　貞憲[1], 矢根　圭[1], 西森博幸[1], 桜井康雄[1], 辻　邦彦[1], 真口宏介[1], 坂田秀勝[2], 松林圭二[2], 加藤俊明[2], 池田久實[2]
手稲渓仁会病院消化器病センター[1], 北海道赤十字血液センター検査部[2]

【背景と目的】近年, 本邦におけるHEVの感染経路や, 臨床像が明らかになりつつある. 今回, 発症前からのviremia推移, 肝炎発症と沈静化までの経過を観察しえた輸血後AH-E 2例を経験したので報告する.
【症例1】60歳代, 男性. 2003年9月NHLymphomaと診断され, 治療中, 血小板減少に対しIr-PCの輸血をうけた. 後日, 輸血されたIr-PCがHEV陽性であることが判明し, 同年10月AH-E発症の可能性が高いと考えられ, 精査加療目的で当院へ転院となる. 輸血21日目にHEV RNA (genotype 4) を検出, 対数増殖期を経て44日目に7.2 log copies/mlとピークを示した. ALTは21日目に正常上限を越え59日目に673 UI/Lまで上昇した. Genotype 4感染による肝炎の重症化を懸念し, IFNを使用したがviremia, 肝炎は改善せず, PSL 30mg内服によりALTは低下, その後抗HEV IgGの出現と同期し87日目にviremiaも改善した.
【症例2】70歳代, 男性. 2005年8月, 急性心筋梗塞治療中に投与されたIr-PCが後にHEV陽性と判明し, AH-E精査加療目的に当院へ転入となる. HEV RNA (genotype 3) は輸血後3日目に同定され, 54日目に7.4 log copies/mlとピークに達し, ALTは17日目に正常上限を越え65日目に972 IU/Lまで上昇した. 抗HEV IgM, IgGが60日目に陽性化後HEV, トランスアミナーゼは低下し, 113日目にviremiaも改善した.
【まとめ】HEV viremiaは10~22日のvirus学的潜伏期間を経て出現し対数増殖後約50日前後にピークを示し, その直後にAST, ALT上昇と血中anti HEVの出現を順に認めた.
【結語】輸血後AH-E2例の全経過を追跡しえた. 本例のように, 発症前から血中virus感染markerを生化学所見, 経過と同時に観察したAH-E例は実験例を除くと未見であり, 貴重な症例と考えられる.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 8. 23	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)		Novitsky V, Gaolathe T, Woldegabriel E, Makhema J, Essex M. Clin Infect Dis. 2007 Sep 1;45(5):e68-71. Epub 2007 Jul 20.	米国

研究報告の概要

○ボツワナにおける血清学検査陰性のHIV-1サブタイプC感染
ボツワナにおける急性HIV-1感染スクリーニング中に特定された抗体陰性のヒト免疫不全ウイルス1型(HIV-1)サブタイプC感染の最初の症例を報告する。HIV-1抗体検査の結果は、迅速検査、通常の酵素免疫測定法及びウエスタンブロットで、全て陰性であった。遺伝子組換えが生じていないHIV-1サブタイプC感染は、ウイルスのgag、pol及びenv遺伝子のジェノタイピングによって確定された。臨床的に安定した状態での患者の紹介からAIDS関連死までの期間は、およそ3ヵ月だった。本症例は、サブタイプCが優勢なアフリカ南部における血清学検査陰性HIV-1感染の発生率を調査することの重要性を示している。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
ボツワナにおいて、HIV-1抗体検査陰性でウイルス遺伝子検査により感染が確認されたHIV-1サブタイプC感染の最初の症例報告である。	日本赤十字社では、HIVについて20プールNATを含むスクリーニングを行い、陽性血液を排除している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

BRIEF REPORT

A Seronegative Case of HIV-1 Subtype C Infection in Botswana

Vladimir Novitsky,[1,2] Tendani Gaolathe,[2] Elias Woldegabriel,[1] Joseph Makhema,[1,2] and M. Essex[1,3]

[1]Botswana–Harvard School of Public Health AIDS Initiative Partnership for HIV Research and Education, and [2]Infectious Disease Care Clinic, Princess Marina Hospital, Gaborone, Botswana; and [3]Department of Immunology and Infectious Diseases, Harvard School of Public Health, Boston, Massachusetts

We report the first case, to our knowledge, of antibody-negative human immunodeficiency virus type 1 (HIV-1) subtype C infection, which was identified during screening for acute HIV-1 infection in Botswana. Results of tests for HIV-1 antibodies were consistently negative, including rapid and regular enzyme-linked immunosorbent assay and Western blot. The nonrecombinant HIV-1 subtype C infection was confirmed by viral genotyping within the *gag*, *pol*, and *env* genes. The period between referral of the patient in a clinically stable condition and AIDS-related death was ~3 months. The reported case indicates the importance of studying the prevalence of seronegative HIV-1 infection in southern Africa, where subtype C predominates.

Routine HIV-1 testing by rapid or standard ELISA has become a commonly used health care practice. The high sensitivity of currently used licensed ELISA kits results in a relatively low frequency of false-negative results during HIV-1 testing. Testing during the seroconversion "window period" is the most recognized reason for a temporarily negative ELISA result; other reasons may include atypical virus or agammaglobulinemia. Although false-negative ELISA results can be confirmed by alternative methods that target viral RNA in plasma and/or cell-associated proviral DNA, the nucleic acid–based methods are not commonly used for routine HIV-1 testing.

Some patients may not develop antibodies after primary HIV infection, and a lack of adequate immune response may facilitate rapid progression of disease [1]. Rare cases of seronegative HIV-1 subtype B infection have been reported in Europe and North America [1–6]. Among non-B clades, HIV-1 subtype A infection was found in a persistently HIV-1–seronegative woman from Ivory Coast [7], subtype A2 virus was identified in an HIV-1–seronegative woman in Portugal [8], HIV-1 CRF01_AE (a circulating recombinant form of HIV-1 that is composed predominantly of subtype A and a unique envelope designated lineage E) was documented in 4 HIV-1–seronegative drug users in Thailand [9, 10], and a rare A/G recombinant was found in an HIV-1–seronegative woman from Ghana [11]. To date, no studies, to our knowledge, have reported cases of seronegative HIV-1 subtype C infection, which is the predominant HIV-1 subtype in the worldwide epidemic.

Here, we report a case of persistently antibody-negative HIV-1 subtype C infection in Botswana. A 46-year-old woman was referred to our study of primary HIV-1 subtype C infection in Botswana for HIV screening with 2 documented antibody-negative rapid ELISA tests performed elsewhere during the previous year. At the time of referral, she did not present with any opportunistic or chronic illnesses but had complaints of recent deterioration in general health and weight loss. The patient had received antifungal treatment for oral candidiasis and nonspecific oral ulcers at the local city-council clinic. No behavioral risk for HIV infection was identified by interview of the patient or review of medical records. She denied having any sexual relationships for >6 months prior to study entrance and reported no blood transfusions or injection drug use.

After receiving counseling and signing an informed consent form, the patient was tested for HIV-1 antibodies and viral RNA. The antibody tests, including rapid ELISA (Determine HIV-1/2, Abbott, and Uni-Gold HIV kit, Trinity Biotech) and regular ELISA (Murex HIV 1.2.0, Abbott, and Ortho HIV-1/2 Ab Capture, Ortho Diagnostics), yielded negative results. Viral RNA was detected in plasma by RT-PCR at >750,000 copies/mL. The tests were repeated, and the results remained the same. Western blot (HIV 2.2 Western Blot assay, Gene Labs Diagnostics) did not reveal any reactive bands. IgG and IgM levels were within the normal range (1410 mg/dL and 179 mg/dL, respectively), whereas the IgA level was elevated to 588 mg/dL. Her $CD4^+$ T cell count was 38 cells/mm^3, and her $CD8^+$ T cell count was 138 cells/mm^3. The patient was offered antiretroviral therapy but declined. Her viral load fluctuated from 451,000 copies/mL to >750,000 copies/mL over 2 months. Her $CD4^+$ cell count decreased to 19 cells/mm^3. The $CD4^+/CCR5^+$ T cell percentage was detected within the range of 10.3%–17.3%, and the $CD4^+/CXCR4^+$ T cell percentage dominated at ~95%.

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Reprints or correspondence: Dr. M. Essex, Dept. of Immunology and Infectious Diseases, Harvard School of Public Health, 651 Huntington Ave., Boston MA 02115 (messex@hsph.harvard.edu).

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Esophageal candidiasis and severe oral herpes were treated with fluconazole and oral acyclovir. Eight days after the initiation of HAART (600 mg of efavirenz once daily and 150 mg of lamivudine plus 300 mg of zidovudine twice daily), the patient presented with inability to communicate and unusual behavior. Altered mental status and HIV encephalopathy were listed as probable clinical conditions. Cryptococcal meningitis and intracranial space-occupying lesions or infections were ruled out by analysis of CSF specimens and CT of the brain. The biochemical laboratory results are summarized in table 1. The patient died 4 days after admission. Encephalopathy or meningitis, HIV infection, and pulmonary tuberculosis were listed as the diseases or conditions leading to death. Immune reconstitution syndrome [12] or herpetic etiology of encephalopathy could not be ruled out. Postmortem examination was not available, and therefore, the cause of death could not be ascertained.

Previous reports of seronegative HIV-1 infections described patients who progressed to AIDS rapidly [1]. Rapid progression was also observed in the current case. Routine identification of antibody-negative HIV infections may be associated with substantial logistical challenges. Nevertheless, initiation of HAART in patients identified to be seronegative who have high plasma viral loads apparently should not be deferred.

It is known that high levels of circulating immune complexes can be found during the late stage of HIV infection. Formation of immune complexes in the sera of patients with AIDS and AIDS-related complex may be responsible for failure to detect free HIV antibodies in some patients [13, 14]. Therefore, we cannot exclude formation of immune complexes as a potential reason for negative results of tests for HIV antibodies in the study patient. However, patients with detectable circulating immune complexes usually have increased levels of each immunoglobulin class [15]. Consequently, the normal levels of IgG and IgM, as well as the results of seronegative tests performed within the year prior to referral, might argue against immune complexes being the main cause of the HIV antibody–negative status of the patient in the current study.

Table 1. Clinical values for a patient presenting with HIV-1 antibody–seronegative AIDS.

Test or variable	Result or value	Reference value
HIV PCR	Positive	...
Rapid plasma regain	Nonreactive	...
Hemoglobin level, g/dL	10.1	12–15
Corpuscular volume, mean fL	78.5	83–99
Neutrophils, %	90.7	40–80
Absolute neutrophil count, $\times 10^9$ neutrophils/L	6.27	2–7
Lymphocytes, %	3.6	20–40
Eosinophils, %	0.0	1–6
Platelet count, $\times 10^9$ platelets/L	280	150–400
Sodium level, mmol/L	126.47	135–145
Potassium level, mmol/L	3.31	3.5–3.1
Chloride level, mmol/L	91.31	95–108
Urea level, mmol/L	6.34	2.0–7.0
Serum creatinine level, μmol/L	77.23	53–97
CSF sample examination		
Glucose level, mmol/L	3.40	2.25–4.55
Total protein level, g/L	1.90	0.10–0.45
Macroscopic examination	Clear, no xanthochromia, no clot	...
Microscopic examination	India ink–negative; no WBCs or yeast cells seen	...
Cryptococcal antigen test	Negative	...
Culture	No growth	...
$CD4^+$ cell count, cells/μL	23.32	700–1000
Viral load, copies/mL	>750,000	...
HIV ELISA	Negative	...
Alkaline phosphatase level	Not determined	...
AST level, U/L	74.86	10–34
ALT level, U/L	16.13	11–44
Hepatitis B virus surface antigen test	Negative	...

NOTE. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

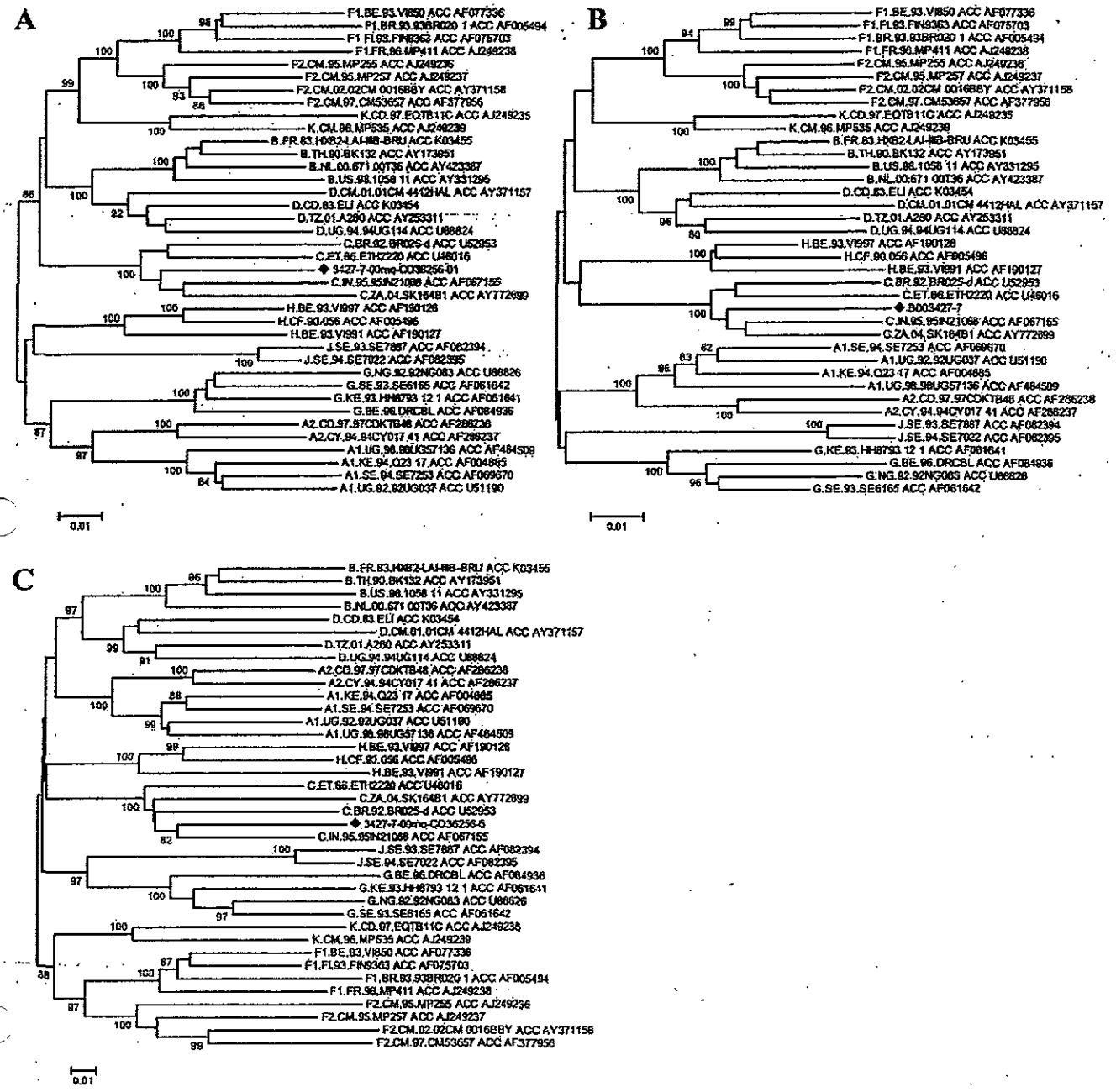

Figure 1. Analysis of phylogenetic relationships between HIV-1 sequences, providing evidence that the sequence from Botswana (indicated by a filled diamond) clusters with HIV-1 subtype C references in *gag (A), pol (B),* and *env (C)* with high bootstrap values. Neighbor-joining trees are presented. Bootstrap values ≥80 are shown.

To address the potential uniqueness of the patient's virus, we performed sequence analysis across structural viral proteins to determine the HIV-1 subtype and to confirm or exclude recombination across the viral genome. The *gag, pol,* and *env* genes were sequenced from the earliest available specimens from the time of referral. A total of 1358 bp was analyzed in *gag;* 1302 bp in *pol,* encoding the entire protease and 435 amino acids of the N-terminal reverse transcriptase; and 1182 bp in *env,* encoding the C2-V5 region of gp120. Phylogenetic analysis demonstrated that the query sequence clustered with HIV-1 subtype C reference sequences within *gag, pol,* and *env* (figure 1). The patient's virus was a typical subtype C virus, which diverged within the expected range from the subtype C consensus and/or ancestral sequence. Detailed sequence analysis did not suggest lack of cross-reactivity between the antigens in the ELISA or Western blot kits. The phylogenetic data provided evidence that a potential mismatch between the HIV-1 antigens in diagnostic kits and in patient's virus is unlikely to account

for the observed seronegativity in the clinical case described here.

Recombinant analysis using the RIP 3 package [16] did not reveal any breakpoints across the analyzed sequences in *gag*, *pol*, and *env* (data not shown). Viral quasispecies were analyzed in *gag* and *env* by single genome sequencing, a new method developed in our laboratory based on a recent report by Palmer et al. [17]. The mean value of *gag* quasispecies nucleotide distance was 6.1% to both HIV-1 subtype C consensus and ancestral sequences. The mean value of *env* quasispecies nucleotide distance was 9.6% to HIV-1 subtype C consensus sequence and 9.9% to HIV-1 subtype C ancestral sequence. Analysis of intrapatient viral diversity revealed a mean value of 0.25% and a median diversity of 0.15% in *gag* and a mean value of 0.6% and a median diversity of 0.55% in *env*. The data obtained strongly suggest that the patient had nonrecombinant HIV-1 subtype C infection with a relatively low level of viral intrapatient diversity at the time of analysis.

In summary, we report the first case, to our knowledge, of antibody-negative HIV-1 subtype C infection. Although the exact time and source of HIV-1 subtype C infection is unknown, it is likely that infection occurred >1 year before death. Repeated tests for HIV-1 antibodies consistently yielded negative results, including rapid and regular ELISA and Western blot. The levels of IgG and IgM were within the normal range, whereas the IgA level was slightly elevated, which excludes agammaglobulinemia as a cause of antibody-negative HIV infection. The nonrecombinant HIV-1 subtype C infection was confirmed by viral genotyping within the *gag*, *pol*, and *env* genes. The period between referral of the patient in a clinically stable condition and AIDS-related death was ~3 months.

This is the first documented case of seronegative HIV-1 subtype C infection. Our findings indicate the importance of future studies to identify the prevalence of seronegative HIV infection in the subtype C epidemic in southern Africa to determine whether viral nucleic acid testing should be introduced in the algorithm of routine HIV-1 screening.

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Potential conflicts of interest. All authors: no conflicts.

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医薬品 研究報告 調査報告

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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)		Editorial team. Euro Surveill. 2007 May 24;12(5):E070524.5.	ヨーロッパ

研究報告の概要

○ヨーロッパにおける供血者のHIV陽性率のモニタリング
AIDS最新号において、LikataviciusらはEuroHIV surveillance network (http://www.eurohiv.org/)によるヨーロッパの供血血液のHIV陽性率についての14年間のモニタリングデータを提示した。この分析は、1990年～2004年までのWHO欧州各国のデータが網羅され、西ヨーロッパ(西欧)、中央ヨーロッパ(中欧)、東ヨーロッパ(東欧)の3地域におけるデータが示されている。2000年～2004年については、1年以上にわたるデータが49カ国から得られた。データの得られた直近の年における平均HIV陽性率は10万供血あたり8.7であり、地域によって大きなばらつきがあった。10万供血あたりの陽性率は、西欧では1.7、中欧では3.4、東欧では36.7であった。スクリーニングを行った供血血液の全てがHIV陰性であったのは11カ国だった。7カ国では、10万供血あたりのHIV陽性率が10以上となった。最も陽性率が高かったのはウクライナで、10万供血あたり128.4だった。経時的な分析によると、供血者のHIV陽性率は1990年以降、西欧で着実に低下し、中欧で横ばい状態であったが、東欧では急激な上昇が認められた(1995年は0.6／10万供血、2004年は40.3／10万供血)。ウクライナでは1995年には陽性率が他国を上回り、2004年まで陽性率は最も高いままだった。この傾向は、これらの地域でのHIV流行を反映しており、東欧の多くの国で特に静注薬物使用者の間でHIVが伝播し、供血を行う可能性のある未診断の新規感染者が多数いることを示している。初回供血者と再来供血者を比較すると、HIV陽性者は供血から除外されるため、一般的に初回供血者の方がHIV陽性率が高いことが、西欧及び中欧20カ国から得られたデータにより示された。供血者集団のHIV陽性率が高い国々における血液供給の安全性が懸念される。当該調査で認められた傾向は、HIVハイリスク者の供血延期や定期供血者プールを維持する等の公衆衛生手段を緊急に実施する必要がある地域を浮き彫りにした。

報告企業の意見	今後の対応
ヨーロッパの供血血液のHIV陽性率についての14年間のモニタリングデータによると、陽性率は西欧、中欧、東欧の順に高くなり、1990年以降の変化では、西欧で低下、中欧で横ばい、東欧では急激な上昇が認められたとの報告である。	日本赤十字社では、HIVについて20プールNATを含むスクリーニングを行い、陽性血液を排除している。国内外のHIV感染、AIDS発生の動向やHIV感染に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

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

Monitoring HIV prevalence in blood donations in Europe

Editorial team (eurosurveillance@ecdc.europa.eu), Eurosurveillance editorial office

In a recent issue of the journal AIDS, Likatavicius *et al.* present data covering 14 years of monitoring the HIV prevalence in blood donations in Europe by the EuroHIV surveillance network (http://www.eurohiv.org/) [1].

The analysis covers data from countries of the World Health Organization (WHO) European Region collected between 1990 and 2004, and presented within three geographic areas: the West, the Centre, and the East*.

For the most recent period, 2000-2004, data for at least one year was provided by 49 countries. In the most recent year with data available, the average HIV prevalence was 8.7 per 100,000 blood donations, with considerable variations between geographical areas and individual countries. The average prevalence in the West was 1.7, in the Centre - 3.4, and in the East - 36.7 per 100,000 blood donations.
In 11 countries, all screened donations tested negative for HIV. In seven countries, the HIV prevalence was more than 10 per 100,000. The highest prevalence was noted in Ukraine, with 128.4 per 100,000 donations that tested positive for HIV.

Analysis of prevalence over time revealed a striking trend: while the prevalence of HIV among blood donations h[illegible] decreased steadily since 1990 in the West and remained stable in the Centre, a dramatic increase was ob[illegible]ved in the East, where the prevalence rose from 0.6/100,000 in 1995 to 40.3/100,000 in 2004. In Uk[illegible]e, the prevalence exceeded that of the remaining countries already in 1995 and continued to do so until 2004.

This trend is a reflection of the development of the HIV epidemic in those regions, with many countries in the East experiencing high ongoing transmission of HIV, in particular among injecting drug users [2], and a large pool of newly infected potential blood donors that have not yet been diagnosed.

The paper also compares first-time donors and repeat donors. Relevant data available from 20 countries in the West and the Centre showed that HIV prevalence among first-time donors was generally higher than among regular blood donors, due to the exclusion of HIV-positive individuals from further donations.

The authors see cause for concern about the safety of the blood supply in countries with a high HIV prevalence among the donor source population. The observed trends highlight areas with an urgent need to implement public health measures such as deferring individuals at high risk of HIV from donating blood and maintaining a pool of regular donors.

* [illegible]ographic areas adopted:

- The West: Austria, Belgium, Denmark, Finland, France (including data from Andorra), Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Malta, Monaco, Netherlands, Norway, Portugal (data from three centres only), San Marino, Spain, Sweden, Switzerland, United Kingdom
- The Centre: Albania, Bosnia and Herzegovina, Bulgaria, Croatia, Cyprus, Czech Republic, Hungary, Macedonia FYR, Poland, Romania, Serbia and Montenegro (excluding data from Kosovo), Slovakia, Slovenia, Turkey
- The East: Armenia, Azerbaijan, Belarus, Estonia, Georgia, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Republic of Moldova, Russian Federation, Tajikistan (no data available for 2000-2004), Turkmenistan (no data available for 2000-2004), Ukraine, Uzbekistan

References:

1. Likatavicius G, Hamers FF, Downs AM, Alix J, Nardone A. Trends in HIV prevalence in blood donations in Europe, 1990-2004. AIDS 2007;21(8):1011-8.
2. Hamers FF, Downs AM. HIV in central and eastern Europe. Lancet 2003;361:1035-44.

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別紙様式第2-1

No. 40

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 20	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)		百瀬俊也. 第31回日本血液事業学会総会; 2007 Oct 3-5; 高松.	日本

研究報告の概要

○感染症リスクと安全対策の取り組み
日本赤十字社血液事業本部が関わる安全対策の取り組みと感染症リスクについて報告する。
平成16年から18年までの3年間に全国の医療機関から日赤血液センターに報告された輸血関連感染症(疑い症例を含む)の報告数は749例であり、その内訳はHBV47.0%、HCV27.6%、細菌21.0%で95.6%を占め、以下、HIV、HEV、ヒトパルボウイルスB19、CMV、HTLV-1、真菌、HAV、単純ヘルペスウイルスと続く。このうち輸血用血液製剤との因果関係が高いと評価した症例は、HBV:37例、HCV:2例、HEV:4例、B19:4例、細菌:3例であった。HBVの37例の内訳は、自発報告19例、遡及調査12例、追跡調査6例であり、HCVの2例は遡及調査で個別NAT陽性となった50プールNATウインドウ期の血液による感染症例であった。HEVについては、4例中3例は北海道で研究的に実施しているHEV-NATにより明らかになった症例である。
一方、献血者の陽性検査結果から始まる遡及調査については、平成11年4月～平成19年3月までの集計で、対象献血件数25,212件の個別NATを実施し、陽性となったのはHBV369件、HCV4件、HIV1件であった。その情報を元に得られた受血者状況は、原疾患等による死亡152件、非陽転80件、陽転22件(内訳:HBV19、HCV2、HIV1)であった。献血後情報の処理件数の9割以上は英国1泊渡航歴によるもので、最盛期(平成17年8月)1,974件／月であったが、平成19年は約350件／月と減少している。
遡及調査及び感染症報告の解析を基礎としたHBVの感染リスクの推計値(年間13～17例)と比較し、過去3年間のHBV感染症例の平均値は12.3例とこれをやや下回る数値となった。日赤の安全対策の実施によりHBV、HCV及びHIVの感染リスクは減少し、安全性は高くなった。しかし、HCV及びHIVも含め遡及調査の実施により確認された感染症例も少なくない。今後、稀な感染症も視野に入れヘモビジランスを推進し、感染拡大を防止するための安全対策を引き続き講じていく必要がある。

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」
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血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本赤十字社血液事業本部が関わる安全対策の取り組みと感染症リスクについての報告である。	日本赤十字社では、HBV、HCV、HIVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)に基づき、輸血感染症の調査を行っている。輸血感染症に関する新たな知見等について今後も情報の収集に努める。次世代NAT試薬についての評価、検査方法の改良に向けた開発・検討を進める。

(二)

30：300　　血液事業 第31回日本血液事業学会総会抄録集 2007.8

シンポジウム4-2

感染症リスクと安全対策の取り組み

日本赤十字社血液事業本部

百瀬俊也

血液事業本部安全管理課が関わる安全対策の取り組みと感染症リスクについて報告する。

平成16年から18年までの3年間に全国の医療機関から日赤血液センターに報告された輸血関連感染症（疑い症例を含む）の報告数は749例であり，その内訳はHBV47.0%，HCV27.6%，細菌21.0%で95.6%を占め，以下、HIV，HEV、ヒトパルボウイルスB19、CMV、HTLV-1，真菌，HAV，単純ヘルペスウイルスと続く。このうち輸血用血液製剤との因果関係が高いと評価した症例は，HBV：37例，HCV：2例，HEV：4例，B19：4例，細菌：3例であった。HBVの37例の内訳は，自発報告19例，遡及調査12例，追跡調査6例であり，HCVの2例は遡及調査で個別NAT陽性となった50プールNATウインドウ期の血液による感染症例であった。HEVについては，4例中3例は北海道で研究的に実施しているHEV-NATにより明らかになった症例である。

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献血後情報の処理件数の9割以上は英国1泊渡航歴によるもので，最盛期（平成17年8月）1,974件／月であったが，平成19年は約350件／月と減少している。

遡及調査及び感染症報告の解析を基礎としたHBVの感染リスクの推計値（年間13～17例）と比較し，過去3年間のHBV感染症例の平均値は12.3例とこれをやや下回る数値となった。日赤の安全対策の実施によりHBV，HCV及びHIVの感染リスクは減少し，安全性は高くなった。しかし，HCV及びHIVも含め遡及調査の実施により確認された感染症例も少なくない。今後，まれな感染症も視野に入れヘモビジランスを推進し，感染拡大を防止するための安全対策を引き続き講じていく必要がある。

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 8. 24	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	CDC Press Release. 2007 Aug 22.	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○CDCの研究者がマールブルグウイルスの感染源と考えられる動物を発見—アフリカフルーツコウモリの感染を特定

米国疾病対策予防センター(CDC)と協力施設の科学者がよく見られるアフリカフルーツコウモリの一種におけるマールブルグウイルスの感染を特定することに初めて成功した。マールブルグウイルスは、ヒトや霊長類に重篤で死に至ることも多い出血熱を引き起こす。コウモリがマールブルグウイルスを保有することが疑われていたが、これまで感染原因となる証拠は検出されていなかった。

「この発見はウイルスと戦う助けになる」とCDCのDr. Julie Gerberdingは話している。「感染経路の特定は一つの課題だった。この研究は、マールブルグウイルスの伝播についてより理解し、ヒトにおける感染拡大を予防・減少させる助力になるだろう」

研究は、ガボン共和国のFrancevilleにある国際医学研究センター(CIRMF)及び開発研究所と共同で行われ、ウイルスの遺伝物質と特異抗体によって特定の種のコウモリにおけるマールブルグウイルス感染が確認された。これは、野生の非霊長類におけるマールブルグウイルス感染の証拠を確認した最初の研究であり、ガボンにおけるこのウイルスの存在を示す最初の報告でもある。この研究では、異なる10種のコウモリ1,100匹以上を検査した。アフリカフルーツコウモリの1種類だけ(*R. aegyptiacus*)がマールブルグウイルス感染陽性となり、他の種からは感染の証拠は見つからなかった。さらに、感染したコウモリから得られたウイルスの遺伝子シークエンスはこれまでに知られている動物やヒト由来のウイルスシークエンスとは異なっていた。このアフリカフルーツコウモリはサハラ以南のアフリカ地域全土で見られる。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
CDCの研究者がよく見られるアフリカフルーツコウモリの一種におけるマールブルグウイルスの感染を特定することに初めて成功したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。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。

(12)

JRC2007T-058

Press Release

For Immediate Release:
Thursday, Aug. 22, 2007

Contact: CDC Division of Media Relations
(404) 639-3286

CDC Researchers find possible animal source for Marburg virus

Identification of infection in a common African fruit bat

Scientists at the Centers for Disease Control and Prevention and their collaborators have for the first time successfully identified Marburg virus infection in a common species of African fruit bat (Rousettus aegyptiacus). Marburg virus causes severe, often fatal, hemorrhagic fever in people and non-human primates. Bats have been suspected of carrying the virus, but until now, evidence of Marburg virus infection in bats had not been detected. These research results were published Wednesday in the open access journal PLoS ONE (www.plosone.org).

"This groundbreaking discovery aids us in our efforts to combat a virus that causes very severe illness," said Dr. Julie Gerberding, CDC director. "One of the challenges has been identifying how people get infected. This research brings us closer to understanding the transmission of Marburg virus and hopefully will help advance our efforts to prevent or reduce the spread of the virus to people. There is much more that we need to learn about hemorrhagic fever viruses, but this is an important step forward."

The work, done in collaboration with the International Center for Medical Research (CIRMF) and the Institute for Research and Development, both in Franceville, Gabon, represents the first time in which Marburg virus genetic material and specific antibodies have both confirmed Marburg infection in a specific bat species. There has been much speculation and scientific investigation of potential reservoirs for Marburg virus, but this is the first study to definitively document evidence of the virus in wild non-primates. It is also the first study to document evidence of Marburg virus in the Central African country of Gabon.

In this study, more than1,100 bats representing 10 different species were tested. Only one species of African fruit bat (R. aegyptiacus), tested positive for Marburg virus infection. No evidence of Marburg virus was identified in the other species of insect-eating or fruit bats tested. Further, viral genetic sequences obtained from the infected bats in this study were unique when compared to other known Marburg virus sequences from animals or humans. This species of African fruit bat is found throughout sub-Saharan Africa.

Marburg virus and the related Ebola virus have caused outbreaks of disease in both people and non-human primates (e.g., African green monkeys), and these outbreaks may result in the death of 80 to 90 percent of those infected. With no vaccine or drug therapy available, outbreaks of Marburg often start abruptly and spread from person-to-person until infection control measures can be implemented. Reports of Marburg hemorrhagic fever are rare, with recent occurrences limited to countries in sub-Saharan Africa.

The publication of this research coincides with a recent investigation of Marburg infection among miners in Uganda. CDC recently deployed a six-person team to investigate the source of the infection, help identify and find people who may have been exposed to the virus or to infected people, and offer guidance on infection control. The team is also working to determine if the same bat species (R. aegyptiacus) or other species of bats might be the source of the infection among the Ugandan miners, and potential routes of transmission between the bats and humans.

"This recent discovery is helping to guide our current outbreak investigation in Uganda," said Dr. Jonathan Towner, lead author of the publication and a member of CDC's Ugandan investigation team. "We're trapping bats that live in the mine to test them for evidence of Marburg virus infection. If infected bats are found, we'll be looking at how they could have transmitted the virus to the miners."

To learn more about Marburg virus please visit: www.cdc.gov/marburg

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DEPARTMENT OF HEALTH AND HUMAN SERVICES

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Page Located on the Web at http://www.cdc.gov/od/oc/media/pressrel/2007/r070822.htm

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医薬品 研究報告 調査報告

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 20	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)		Schilling S, Emmerich P, Klempa B, Auste B, Schnaith E, Schmitz H, Kruger DH, Gunther S, Meisel H. J Clin Microbiol. 2007 Sep;45(9):3008-14. Epub 2007 Jul 11.	ドイツ

研究報告の概要

○ドイツのハンタウイルス感染症アウトブレイク:ルーチンの血清学的検査の限界とヒトおよびげっ歯類由来のウイルス配列の集積
ヨーロッパでの出血熱症候群は、主にPuumalaウイルス(PUUV)またはDobravaウイルスへの感染によるものである。ドイツ南東部バイエルン(バヴァリア)南部でのハンタウイルス感染症アウトブレイクの患者31名について、酵素免疫測定法、免疫蛍光検査法、免疫ブロット解析血清学的検査を行った。しかし、これらの症例のうちの数名は、標準的検査による抗体のPUUV特異的タイピングが成功せず、当該ウイルスタイプによる感染を確定するのにバイオセーフティーレベル3でのウイルス中和測定を必要とした。3名の患者の急性期血清から得たPUUV RNAをRT-PCR を用いて増幅したところ、同地域で捕獲したハタネズミ (Clethrionomys glareolus) 3匹から得たウイルス配列と非常に近縁であることを認めた。以上の知見は、当該アウトブレイクと現地げっ歯類集団に蔓延する新規PUUV"バイエルン"系統とを関連づけるものである。当該アウトブレイクに関与するバイエルン系統は、ドイツの他のPUUV系統とは遠縁でしかない。

報告企業の意見	今後の対応
ドイツ南東部バイエルン(バヴァリア)南部でのハンタウイルス感染症のアウトブレイク時に、一部の患者から同地域のハタネズミから得たウイルス配列と近縁のウイルスが検出され、当該アウトブレイクと現地げっ歯類集団に蔓延する新規PUUVバイエルン系統とが関連づけられたとの報告である。	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。

使用上の注意記載状況・その他参考事項等

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Hantavirus Disease Outbreak in Germany: Limitations of Routine Serological Diagnostics and Clustering of Virus Sequences of Human and Rodent Origin▽

Stefan Schilling,[1]† Petra Emmerich,[1] Boris Klempa,[2] Brita Auste,[2] Ebbo Schnaith,[3] Herbert Schmitz,[1] Detlev H. Krüger,[2]* Stephan Günther,[1] and Helga Meisel[2]

Department of Virology, Bernhard-Nocht-Institute for Tropical Medicine, Hamburg,[1] Institute of Virology, Helmut-Ruska-Haus, Charité University Hospital, Berlin,[2] and Laboratory Schubach and Associates, Passau,[3] Germany

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In Europe, hemorrhagic fever with renal syndrome results mainly from infection with Puumala virus (PUUV) or Dobrava virus. For 31 patients from a hantavirus disease outbreak in Lower Bavaria, a district in southeast Germany, serodiagnosis was undertaken by enzyme-linked immunosorbent assay, immunofluorescence assay, and immunoblot analysis. In a few of these cases, however, PUUV-specific typing of antibodies by these standard assays failed and a virus neutralization assay under biosafety level 3 conditions was required to verify the infection by this virus type. PUUV RNA was amplified by reverse transcription-PCR from acute-phase sera of three patients and was found to be very closely related to virus sequences obtained from bank voles (*Clethrionomys glareolus*) trapped in the same area. These findings link the outbreak with a novel PUUV lineage, "Bavaria," circulating in the local rodent population. The Bavaria lineage associated with the outbreak is only distantly related to other PUUV lineages from Germany.

Two human zoonoses, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (also named hantavirus pulmonary syndrome) are caused by infections with hantaviruses. Case fatality rates can reach up to 50%. The agents, negative-strand RNA viruses forming the genus *Hantavirus* within the *Bunyaviridae* family, are transmitted to humans from their respective rodent reservoirs (8, 13, 19). In Central Europe, HFRS occurs mainly due to infections by Puumala virus (PUUV) transmitted from the red bank vole (*Clethrionomys glareolus*) and by a Dobrava virus (DOBV) lineage, named DOBV-Aa, carried by the striped field mouse (*Apodemus agrarius*) (7, 8, 10, 23). Infections by both PUUV and DOBV-Aa usually cause mild to moderate HFRS, which is also known as nephropathia epidemica (22). Germany reports up to 450 HFRS cases per year (18).

Since viremia in HFRS patients is short termed and characterized by fluctuating RNA levels, molecular genetic approaches are not common in the identification of human infections. Instead, laboratory diagnosis is based on serology. Usual methods are enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) formats based on the immunodominant nucleocapsid protein (N) antigen and virus-infected cells, respectively, to detect antibodies of immunoglobulin M (IgM) and IgG classes. Also, an immunoblot assay, based on the use of N antigen, has been established. The majority of hantavirus-infected patients present detectable IgM class antibodies already at the onset of disease. In only few cases was the IgM response found to be delayed. IgM disappears within months; however, when using sensitive test formats, it can be detected in single cases as late as 2 years after the acute phase of infection (8, 12). The IgG response is long lasting but sometimes delayed; in about 10% of acute-phase, IgM-positive sera, no IgG can be found with routine assays (8, 20).

Here we describe the serological and molecular epidemiological investigation of an outbreak of hantavirus disease in Lower Bavaria, a district in southeast Germany (Federal State of Bavaria), in 2004. We tested early and, in some cases, follow-up sera of 31 patients, showing that the outbreak was caused by Puumala virus. In certain cases, ELISA, IFA, or immunoblot assays were unable to support the diagnosis of PUUV infection because of extended cross-reaction of IgM and even IgG antibodies to other hantavirus antigens. In these cases, focus reduction neutralization testing (FRNT) confirmed PUUV infection. PUUV sequences were amplified from sera from three patients and compared with those from bank voles trapped in the same area where those three patients resided. The human infections were caused by a novel genetic lineage of the PUUV species, called Bavaria.

MATERIALS AND METHODS

Patients and sera. Serum samples were collected by local physicians from patients with clinical signs of HFRS (nephropathia epidemica) and sent to the German Reference Centers for Tropical Infections (Hamburg) and Hantavirus Infections (Berlin) for diagnosis. First serum samples were taken during the acute phase (n = 31). In some cases, a second sample (n = 13) was collected during convalescence.

Rodent trapping. Rodents were trapped at Bavarian National Park (village of Neuschönau) located in the vicinity of the sites where human infections occurred. Pleural and abdominal fluids, lungs, spleens, kidneys, and urinal bladders were stored at −70°C.

Serological assays. All human serum samples and body fluids of rodents were tested for IgG and IgM antibodies to PUUV, DOBV, or Hantaan virus (HTNV)

* Corresponding author. Mailing address: Institut für Medizinische Virologie, Helmut-Ruska-Haus, Charité—Universitätsmedizin Berlin, Charitéplatz 1, D-10117 Berlin, Germany. Phone: 49-30-450 525 092. Fax: 49-30-450 525 907. E-mail: detlev.kruger@charite.de.

† Present address: Center of Internal Medicine, Division of Infectious Diseases, University Hospital Frankfurt/Main, 60590 Frankfurt/Main, Germany.

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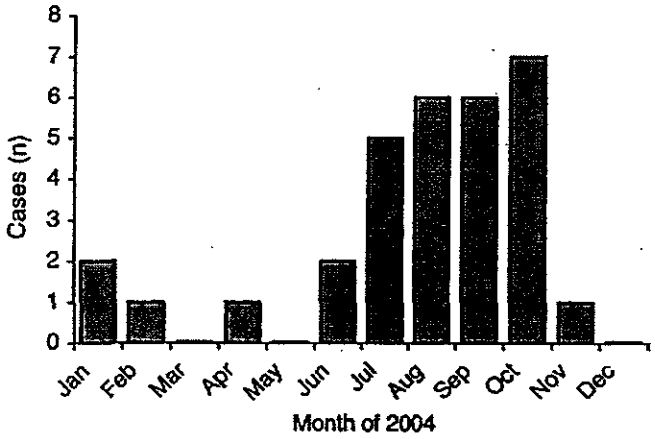

FIG. 1. Epidemic curve based on the 31 cases of laboratory-confirmed HFRS in a region in Lower Bavaria, Germany.

by IFA and ELISA. IgG and IgM IFA were performed with HTNV (strain A549/76-118)- and PUUV (strain B1NE 568/8)-infected Vero E6 cells. Cells were spread onto slides, air dried, and acetone fixed. Samples were serially diluted in twofold steps in phosphate-buffered saline starting with an initial dilution of 1:10, added to the cells, and incubated for 90 min at 37°C. After washing in phosphate-buffered saline, slides were incubated with fluorescein isothiocyanate-labeled rabbit anti-human IgG and IgM antibodies (which are gamma-chain and mu-chain specific, respectively; SIFIN, Berlin, Germany) at 37°C for 25 min. For rodent samples, fluorescein isothiocyanate-labeled goat anti-mouse IgG/IgM (SIFIN, Berlin, Germany) was used. IgG titers of 1:40 or more and IgM titers of 1:20 or more were considered positive.

IgG and IgM ELISA was performed using recombinant nucleocapsid protein of PUUV, DOBV, and HTNV as described previously (12). Titers of 1:400 or more were considered positive. Selected sera were tested using commercially available Western blots (recomBlot; Mikrogen, Martinsried, Germany) according to the manufacturer's instructions and by FRNT. FRNT was performed with PUUV, DOBV, and HTNV under biosafety level 3 conditions as described previously (5).

Reverse transcription-PCR (RT-PCR), cloning, and sequencing. For the isolation of RNA from patient sera, the QIAamp viral RNA mini kit (QIAGEN, Hilden, Germany) was used. Serum (140 μl) was added to 560 μl of buffer AVL with carrier RNA, and the standard QIAamp viral RNA mini spin protocol was performed. RNA was eluted to 60 μl of buffer AVE.

Total cellular RNA of seropositive rodents was extracted from homogenized lung and kidney tissues by using TRIzol reagent (GibcoBRL, Invitrogen, Karlsruhe, Germany) according to the manufacturer's protocol. RNA was reverse transcribed with murine leukemia reverse transcriptase (Invitrogen, Karlsruhe, Germany) by using random hexamers. PUUV-specific nested PCR was performed as described previously (1) using the TEMPase hot-start master mix kit (BIOMOL, Hamburg, Germany). In addition, overlapping fragments were generated by two seminested PCRs to obtain the complete nucleotide sequence of the first PCR product.

The amplified products were cloned into pCR 2.1 TOPO vector (TA cloning kit; Invitrogen, Leek, Netherlands). At least three recombinant plasmids were sequenced in both directions, and the consensus sequence was determined. Dideoxy sequencing was performed on a LICOR sequencer using the AutoRead kit (Pharmacia-Biotech, Freiburg, Germany) as described by the manufacturer.

Sequence and phylogenetic analyses. The sequence data were further analyzed using the BioEdit software package (4). Sequences were aligned using the CLUSTALW algorithm (25). The reliability of the alignment was checked using DotPlot analysis implemented in the BioEdit software package. The alignment was tested for phylogenetic information by likelihood mapping analysis (24) implemented in the TREE-PUZZLE 5.2 software program (21). A neighbor-joining phylogenetic tree was constructed using the PAUP* software program. The Tamura-Nei evolutionary model (TN93) was used to calculate genetic distances. Bootstrap statistical support values were calculated on 10,000 bootstrap replicates.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this study were deposited in GenBank under accession numbers EU004029 to EU004036.

RESULTS

Epidemiology of the outbreak. The region around Passau in Lower Bavaria was not a hot spot of hantavirus transmission in Germany before 2004. In the previous years, no cases to three cases per annum were reported from the same district (17). In contrast, between January and November 2004, we confirmed acute hantavirus infection in 31 patients (mean age, 36 years; 78% male) by laboratory investigation. Until May 2004, four sporadic infections occurred. The peak of the epidemic was observed between June and October with 27 cases. The epidemic curve suddenly declined in November (Fig. 1). Most of the cases occurred in a radius of about 25 km in a region of the Bavarian Forest north of the city of Passau (Fig. 2).

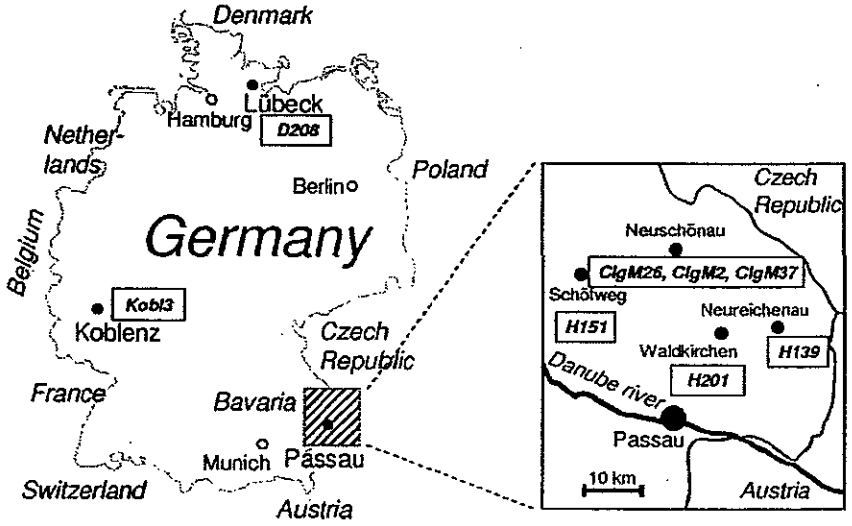

FIG. 2. Map of Germany and the outbreak region in Lower Bavaria (Germany). Black circles indicate the places of residence of three patients (H139, H151, and H201) and trapping places of rodents (ClgM2, ClgM26, and Clg201 in Lower Bavaria; Kobl3 in west Germany; and D208 in north Germany) from which hantavirus RNA was recovered.

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TABLE 1. Serological testing of samples from HFRS patients using different assays

Virus antigen[a] and serological assay	Value for serum sample			
	Acute phase (n = 31)		Convalescent phase (n = 13)	
	% Positive	Reciprocal GMT[b]	% Positive	Reciprocal GMT[b]
PUUV				
IgM ELISA	100.0	1,796	92.3	1,425
IgM IFA	93.8	107	69.2	93
IgG ELISA	93.8	5,489	100.0	20,683
IgG IFA	100.0	2,772	100.0	7,050
HTNV				
IgM ELISA	43.8	635	76.9	606
IgM IFA	87.5	40	69.2	43
IgG ELISA	56.3	491	100.0	1,980
IgG IFA	96.9	162	100.0	198
DOBV				
IgM ELISA	62.5	481	53.8	487
IgG ELISA	81.3	1,204	100.0	1,780

[a] N antigen for ELISA; virus-infected cells for IFA.
[b] GMT of positive samples.

All patients met the clinical criteria of nephropathia epidemica (8), and most of them required hospitalization due to impaired renal function. Neither severe hemorrhagic complications nor fatal courses of infection were observed.

Since southeast Germany is a geographical region where *Clethrionomys glareolus*, but not *Apodemus agrarius*, is prevalent as a hantavirus reservoir, PUUV could be expected as the etiological agent of the outbreak.

Serological investigation of patient sera. Serum samples from the acute phase of illness were available from 31 patients. A follow-up serum sample was collected 1 to 20 weeks later from 13 of the 31 patients. All 44 sera were tested for IgM and IgG antibodies by IgM and IgG ELISA, respectively, on the basis of PUUV, DOBV, and HTNV nucleocapsid protein antigens and by IFA using PUUV- or HTNV-infected cells. Because HTNV is antigenetically closely related to DOBV (8) and HTNV-infected cells were immediately available, they were used to detect DOBV-specific antibodies by IFA.

The largest number of positive samples and the highest reciprocal geometric mean titer (GMT) of positive samples was seen in the PUUV-specific assays, suggesting that the outbreak was caused by PUUV (Table 1). All acute-phase sera were IgM positive by PUUV-specific ELISA. The percentage of IgM-positive samples and their GMTs were lower by IFA than by ELISA. Similar differences were observed for the follow-up samples, suggesting that IFA is less sensitive than ELISA for IgM detection. No major differences were seen between the two methods with respect to IgG detection.

However, in a few cases, IgM titers against heterologous DOBV and HTNV antigens were similar (by IFA) or even higher (by ELISA) (Table 2). Antibody specificity in these sera was further tested by immunoblot analysis. In all cases but one, higher IgM reactivity against PUUV was demonstrated (Table 2).

Table 3 summarizes results for four sera with indeterminate or DOBV-/HTNV-specific IgG typing by ELISA. All sera reacted with the highest titers against PUUV by IFA, whereas immunoblot analysis identified dominant PUUV reactivity only in serum sample 361. Immunoblot results for serum samples 2395 and 2813 corresponded to the indeterminate typing results by ELISA. In the case of serum sample 2395, the blot analysis of a consecutive serum sample (sample 2753) from the same patient showed a slight PUUV antigen preference.

Due to the particularly conflicting IgG results for serum samples 361 and 2813 by ELISA, immunoblot analysis, and IFA, we decided to use FRNT to determine the specificity of the sera. The typing of neutralizing antibodies by FRNT is the method of choice to determine the hantavirus species that led to the infection of a patient (8). Table 4 demonstrates that serum samples 361 and 2813 neutralized PUUV clearly better than they neutralized DOBV or HTNV, strongly suggesting that the corresponding patients had been infected by PUUV.

Testing of patient sera by PUUV RT-PCR. RNA was extracted from all sera and tested by PUUV S segment-specific RT-PCR. Genetic material was amplified from only three sera taken early after the onset of disease. The three patients (designated H139, H151, and H201) were living in different villages in the Bavarian Forest north of the city of Passau (Fig. 2). All three cases were characterized by acute onset with fever, myalgia, back pain, and abnormal fatigue. Acute renal failure was accompanied by thrombocytopenia and an increase in serum creatinine. Hemodialysis treatment was not necessary, and all three patients recovered. Interestingly, in one of the three patients (H151), blurred vision, conjunctival petechiae, and lung

TABLE 2. Exceptional results of IgM assays without clear PUUV preference[a]

Serum no.	Result for indicated antigen by IgM								
	ELISA			IFA		Immunoblot			
	PUUV	DOBV	HTNV	PUUV	HTNV	PUUV	DOBV	HTNV	SEOV
235	**400**	**1,600**	**6,400**	**160**	**160**	++	–	–	–
236	**1,600**	**1,600**	**1,600**	**160**	**160**	++	–	–	–
2395	6,400	1,600	0	**20**	**20**	+	+	+	+
3128[b]	**25,600**	1,600	**25,600**	>640	80	++	–	–	–
3156	**1,600**	**1,600**	**1,600**	80	20	++	–	–	–
3300[c]	**1,600**	**1,600**	400	320	80	++	–	–	–

[a] ELISA, IFA, or blot data not directly supporting the diagnosis of PUUV infection are shown in bold. –, no reaction; +, same intensity as the cutoff band; ++, strong intensity (stronger than the cutoff band); SEOV, Seoul virus. Numbers are reciprocal titers.
[b] RT-PCR data available.
[c] Sampled from the same patient as that for serum sample 3156 but obtained 2 weeks later.

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TABLE 3. Exceptional results of IgG assays without clear PUUV preference[a]

Serum no.	Result for indicated antigen by IgG								
	ELISA			IFA		Immunoblot			
	PUUV	DOBV	HTNV	PUUV	HTNV	PUUV	DOBV	HTNV	SEOV
361	**25,600**	**102,400**	400	2,560	640	+++	(+)	−	−
2395	**25,600**	**25,600**	ND	2,560	80	++	++	++	+
2753[b]	**25,600**	**25,600**	**25,600**	20,480	320	+++	++	++	+
2813	**1,600**	**25,600**	**25,600**	5,120	160	+	+++	+++	++

[a] ELISA, IFA, or blot data not directly supporting the diagnosis PUUV infection are shown in bold. −, no reaction; (+), intensity weaker than the cutoff band; +, same intensity as the cutoff band; ++, strong intensity (stronger than the cutoff band); +++, very strong intensity; SEOV, Seoul virus; ND, not determined. Numbers are reciprocal titers.

[b] Sampled from the same patient as as that for serum no. 2395 but obtained 1 month later.

infiltration were observed during the first days of disease. The sequences of the PCR products revealed infection by a specific PUUV strain (see below), confirming the serological findings and providing direct evidence that PUUV caused the epidemic.

Rodent trapping and analysis. To investigate whether the PUUV strain identified in the patients was also prevalent in the local rodent population, 38 bank voles were trapped in November 2004 in the outbreak region, National Park "Bavarian Forest" (Fig. 2). Seven of 38 animals (18.4%) were found seropositive for PUUV by IFA. Eight animals, including two seronegative voles, were positive by PUUV S segment RT-PCR analysis of lung and kidney tissues. The amplified PCR products from the rodents were sequenced and used for molecular epidemiological analysis.

Furthermore, we wondered whether the PUUV strain is specific to the Bavarian region. Therefore, rodents trapped in other regions of Germany were included in the study. Three of 49 bank voles (6.2%) trapped in August 2004 in northeast Germany near the city of Lübeck and five of 16 bank voles (31.25%) trapped in June 2005 in West Germany near the city of Koblenz tested positive by PUUV-specific RT-PCR. The amplified DNA fragments were sequenced and included in the phylogenetic analysis.

Sequence and phylogenetic analyses. Partial S segment sequences (616 nucleotides, positions 354 to 969) of PUUV from sera of the three patients (designated strains H139/04, H151/04, and H201/04), kidney tissues of three bank voles trapped in the outbreak region (designated strains ClgM2/04, ClgM26/04, and ClgM37/04), and kidney tissues of two bank voles trapped outside the outbreak region (designated strains D208/04 [from the Lübeck region in North Germany] and Kobl3/05 [from the Koblenz region in West Germany]) (Fig. 2) were subjected to sequence comparisons and phylogenetic analysis.

The sequence analysis showed that all human and rodent sequences from the outbreak region were very closely related, with 97.4 to 99.0% sequence identity. Altogether, there were 32 polymorphic sites within the alignment of the Bavarian sequences. Only one substitution represents a nonsynonymous mutation leading to the conservative amino acid exchange I251M in the putative N protein, where the strains H139/04 and ClgM26/04 encode isoleucine (I) but the other Bavarian strains carry methionine (M) (data not shown). Sequence similarity of the Bavarian strains to other German and European PUUV sequences was rather low (81.4 to 87.0%). The only German PUUV (partial) nucleotide sequence recovered from a patient with HFRS so far (15) showed only 84.7 to 85.3% similarity to the nucleotide sequence of the Berkel strain (accession no. L36943). Interestingly, the most related sequence is not from a German strain but from strain Opina-916 (accession no. AF294652) obtained from a bank vole captured in Slovakia (9).

Phylogenetic analysis (neighbor joining) of 60 PUUV sequences confirmed the clear molecular epidemiological link between PUUV strains from patients and rodents collected in the outbreak area. All Bavarian sequences formed a well-supported monophyletic group, indicating that the outbreak had been caused by a specific Bavarian lineage of PUUV that differs from lineages found in other parts of Germany. Even a direct coupling of patient- and rodent-associated sequences could be observed in the cases of H139/04 and ClgM26/04 sequences (Fig. 3).

As expected, all analyzed German sequences clustered within the Central European clade formed by strains from Belgium, Slovakia, and Germany. Within this clade, at least four lineages can be distinguished further. The strains from Bavaria form one of them. The strain from Slovakia represents the second lineage. The third group is formed by strains from Belgium and northwest Germany (Cg-Erft, accession no. AJ238779) and by our strains Kobl3/05 from west Germany and D208/04 from north Germany. The fourth lineage is represented by another sequence from northwest Germany (Berkel, accession no. L36943) obtained from a patient with severe HFRS (15). Interestingly, the statistical support for the monophyletic group of the three above-defined lineages (bootstrap value of 88 [data not shown]) was removed by the addition of the latter sequence into the data set.

TABLE 4. Typing of selected sera by FRNT

Serum no.	Reciprocal endpoint dilution of serum neutralizing:		
	PUUV	DOBV	HTNV
361	2,560	40	160
2813	640	<40	160

DISCUSSION

Here we report the serological and molecular epidemiological investigation of an outbreak of hantavirus disease involving 31 patients in southeast Germany. The patients exhibited clinical symptoms and biochemical findings typical of HFRS cases

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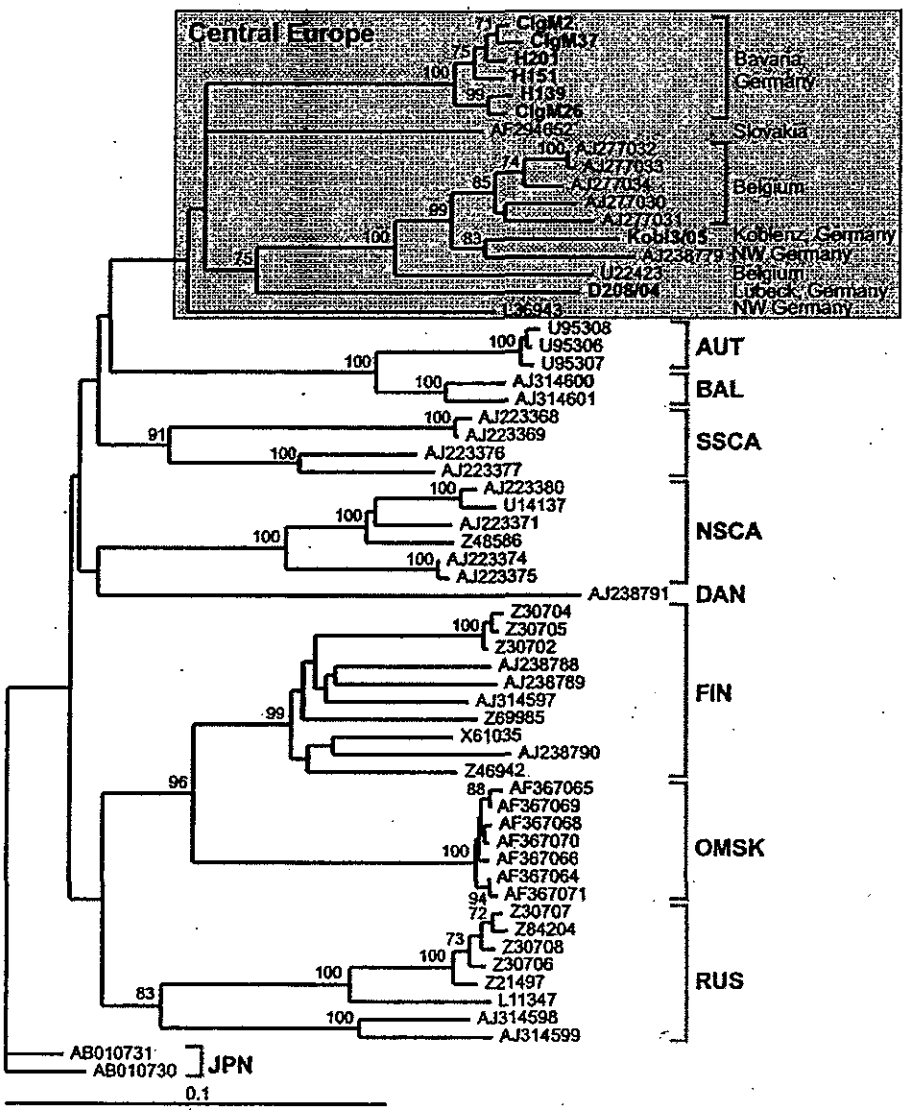

FIG. 3. Neighbor-joining phylogenetic tree (TN93 evolutionary model) of European PUUV strains based on S segment partial sequences (616 nucleotides, positions 354 to 969). Bootstrap values higher than 70, calculated from 10,000 replicates, are shown at the tree branches. PUUV-like sequences from Japan were used as an outgroup. All sequences recovered from GenBank are indicated by their accession numbers. Sequences covered within this study are shown in bold; their GenBank accession numbers are EU004029 (H139), EU004030 (H151), EU004031 (H201), EU004032 (ClgM2), EU004033 (ClgM26), EU004034 (ClgM37), EU004035 (D208), and EU004036 (Kobl3). Previously defined lineages are indicated by abbreviated names. AUT, Austrian; BAL, Balkan; DAN, Danish; FIN, Finish; JPN, Japanese; NSCA, North Scandinavian; OMSK, Russian from Omsk region; RUS, Russian; SSCA, South Scandinavian.

occurring in Germany. The findings show that the outbreak was caused by a novel strain of PUUV that was only distantly related to other German PUUV strains.

In most cases, serological testing identified PUUV as the causative agent. In contrast to the common view, standard methods such as ELISA, IFA, and immunoblot analysis were not always able to determine the causative hantavirus species. In these cases, the testing of consecutive sera or typing of neutralizing antibodies by FRNT was needed to confirm the virus-specific diagnosis of PUUV infection. Our findings demonstrate that due to the cross-reactivity of sera, serotyping for diagnoses of individual patients as well as seroepidemiological studies may sometimes not correctly identify the causative hantavirus if typing is based only on ELISA, IFA, or immunoblot analysis.

Besides the neutralization assay, RT-PCR and sequencing provide clear information on the causative virus species. However, it is known that PUUV infection leads to a short and fluctuating viremia. This is why the recovery of viral nucleic acid sequences from patient sera is possible during only the first days after the onset of disease and may even fail during this time. We were able to amplify viral sequences from early sera of three patients. Sequence analysis confirmed the diagnosis of PUUV infection and indicated that the outbreak was caused by a novel virus strain.

The trapping of bank voles in the vicinity of human cases

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allowed us to compare nucleotide sequences of human and rodent origin from the hantavirus disease outbreak. Virus sequences from patients were phylogenetically closely related to those of the bank voles, suggesting that the patients had been infected by the prevalent PUUV strains circulating in this area. This view is supported by an independent study, which was focused on the reservoir species in the outbreak area (3). All sequences obtained from these animals were also closely related to the human sequences determined in our study (up to 100% identity).

In the analyzed S segment region, we found a degree of informative nucleotide substitutions of 2.1% between the Bavarian sequences of human and rodent origin accompanied by one amino acid difference. In general, we and others (6, 16) did not see specific mutations that can be associated with the adaptation of a rodent-derived hantavirus to a human host. Probably, such a mutation(s) is not necessary as long as the infected patient is a "dead-end host" for the virus and a complete adaptation to the new host with high viraemia, enabling its human-to-human transmission, does not occur. There are only a few reports about interhuman transmission of Andes hantavirus; however, no comparison between viral sequences from these persons and their rodent sources of infection has been undertaken (11, 14).

In addition to the PUUV strains from the outbreak in Bavaria, we included novel PUUV sequences from North and West Germany in the phylogenetic analysis in order to determine the evolutionary origin of the outbreak strain in more detail and to expand our view about the molecular diversity of PUUV circulating in Central Europe (Fig. 3). To our surprise, the Bavarian lineage did not cluster with the German strains from Koblenz and Lübeck but was equidistant from the Slovakian PUUV on the one hand and the German and Belgium strains on the other hand. Thus, the phylogenetic reconstruction suggests the existence of at least two well-supported Central European lineages of PUUV (Bavaria; Belgium/NW Germany). The strains AF294652 (Slovakia) and L36943 (NW Germany) may occupy separate lineages but also may join one of the supported lineages; the exact phylogenetic relationship between these lineages could not yet be resolved on the basis of the existing sequence information. Strains from another Central European country, Austria (1), belong to a different phylogenetic clade, together with southeast European strains from the Balkans (Fig. 3).

The reason for the outbreak of hantavirus disease in the Bavarian Forest in 2004 is not known. The local accumulation of cases is remarkable since, in Germany, the number of recorded HFRS cases had not increased compared with the annual average. One might speculate that 2004 was "high mast year" for *Clethrionomys glareolus* animals in this wooded area, although underlying ecological changes are unknown. A higher rodent density facilitates the spreading of virus between animals and its transmission to humans. Similar reasons were assumed to explain the first outbreak of hantavirus cardiopulmonary syndrome in the southwestern United States in 1993 (2). In addition, information in the local press in the Bavarian Forest area at the beginning of the hantavirus outbreak could have increased the awareness of physicians of this disease and might have contributed to the high number of recorded HFRS cases.

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研究報告の概要

呼吸器感染は、最も広範なヒトの感染症を構成しており、その相当な割合が未知の病原体によって引き起こされている。レオウイルス（respiratory enteric orphan viruses）は、1950 年代初めにヒトから初めて分離され、それまでに知られている如何なる疾患とも関係していなかったことから、そう名付けられた。ここで我々は、マレーシアの Melaka において 39 歳の男性患者から分離された、以前には知られていなかったレオウイルス（Melaka ウイルスと名付けられた）について報告するが、患者はウイルス分離の際、高熱と急性呼吸器疾患に苦しんでいた。患者の家族の内の 2 名が 1 週間以内に同様の症状を呈し、同じウイルスに感染していることを示す血清学的証拠を有していた。
疫学的追跡調査により、その父親が臨床的症状を発現する 1 週間以内に、その家族がコウモリに曝露されていたことが明らかとなった。遺伝子配列分析によって、1999 年にマレーシアの Tioman 島のオオコウモリから分離されたレオウイルスである Pulau ウイルスと Melaka ウイルスの間に、緊密な遺伝子的関係があることが示された。この島のボランティアからの血清のスクリーニングから、109 人中 14 人（13%）が Pulau と Melaka の両ウイルスに陽性であることが明らかとなった。これは、ヒトの急性呼吸器疾患と関係したオルソレオウイルスについての最初の報告である。Melaka ウイルスは、ヒトに無症候性に感染することが知られている哺乳動物の種々のレオウイルスとは血清学的に関係していない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
ヒトの急性呼吸器疾患と関係したオルソレオウイルスについての最初の報告である。 Melakaウイルスはレオウイルス科に属し、エンベロープを有しない、直径69～84nmのRNAウイルスである。これまで血漿分画製剤によってMelakaウイルスが伝播したとの報告はない。原料血漿にMelakaウイルスは混入し得るのか否か、混入する場合どの程度混入し得るのか等の情報が得られていないため、現時点では安全性評価は困難であり、今後も情報収集に努める。	Melaka ウイルスに関連する情報については、今後も注視することとする。

A previously unknown reovirus of bat origin is associated with an acute respiratory disease in humans

Kaw Bing Chua*†, Gary Crameri‡, Alex Hyatt‡, Meng Yu‡, Mohd Rosli Tompang*, Juliana Rosli*, Jennifer McEachern‡, Sandra Crameri‡, Verasingam Kumarasamy*, Bryan T. Eaton‡, and Lin-Fa Wang‡†

*National Public Health Laboratory, Selangor 4700, Malaysia; and ‡Commonwealth Scientific and Industrial Research Organization Livestock Industries, Australian Animal Health Laboratory and Australian Biosecurity Cooperative Research Center for Emerging Infectious Diseases, Geelong, Victoria 3220, Australia

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Respiratory infections constitute the most widespread human infectious disease, and a substantial proportion of them are caused by unknown etiological agents. Reoviruses (respiratory enteric orphan viruses) were first isolated from humans in the early 1950s and so named because they were not associated with any known disease. Here, we report a previously unknown reovirus (named "Melaka virus") isolated from a 39-year-old male patient in Melaka, Malaysia, who was suffering from high fever and acute respiratory disease at the time of virus isolation. Two of his family members developed similar symptoms ≈1 week later and had serological evidence of infection with the same virus. Epidemiological tracing revealed that the family was exposed to a bat in the house ≈1 week before the onset of the father's clinical symptoms. Genome sequence analysis indicated a close genetic relationship between Melaka virus and Pulau virus, a reovirus isolated in 1999 from fruit bats in Tioman Island, Malaysia. Screening of sera collected from human volunteers on the island revealed that 14 of 109 (13%) were positive for both Pulau and Melaka viruses. This is the first report of an orthoreovirus in association with acute human respiratory diseases. Melaka virus is serologically not related to the different types of mammalian reoviruses that were known to infect humans asymptomatically. These data indicate that bat-borne reoviruses can be transmitted to and cause clinical diseases in humans.

respiratory infection | zoonosis | human-to-human transmission | orthoreovirus | Pulau virus

Respiratory tract illness (RTI) accounts for a large portion of public health spending worldwide. Although several new etiological agents of RTI have been identified in the past few decades, a substantial proportion of these illnesses remain undiagnosed. Recently, three previously unknown coronaviruses have been identified as the causative agents for mild to very severe RTI, including the novel coronavirus responsible for the global outbreak of the severe acute respiratory syndrome (SARS) (1–3).

Reoviruses (respiratory enteric orphan viruses) are a large and diverse group of nonenveloped viruses with segmented dsRNA genomes that are taxonomically classified into 10 genera in the family *Reoviridae* (4, 5). Members of the genus *Orthoreovirus* contain 10 genome segments and have been isolated from a broad range of mammalian, avian, and reptilian hosts. Orthoreoviruses are divided into two subgroups, fusogenic and nonfusogenic, based on the ability of the virus to induce cell–cell fusion and syncytium formation (6, 7). The mammalian orthoreoviruses (MRV) are nonfusogenic, whereas the remaining members of the genus are fusogenic, including avian orthoreoviruses, baboon orthoreoviruses, reptilian orthoreoviruses, and Nelson Bay orthoreovirus (NBV). Since the first isolation of MRV from humans in 1951, it has been shown that MRV infection is quite common in the human population (8). However, although many diseases in animals have been attributed to orthoreovirus infection, from neurological symptoms in baboons and snakes to pneumonia and death in chickens, infections in humans are generally benign with very rare cases of mild upper respiratory tract illness or enteritis in infants and children (7).

Bats, probably the most abundant, diverse, and geographically dispersed vertebrates on earth, have recently been shown to be the reservoir hosts of a variety of zoonotic viruses responsible for severe human disease outbreaks, some with very high mortality (9). In the period from 1994 to 1999, four new viruses in the family *Paramyxoviridae* were discovered, and all appeared to have bats as a reservoir host. Hendra virus emerged in Queensland, Australia, in 1994, killing one human and 14 horses (10), and was responsible for at least four other sporadic outbreaks involving horse and human cases between 1994 and 2006 (11). The closely related Nipah virus (NiV) emerged in 1998–1999 in Peninsular Malaysia, resulting in the death of >100 people and the culling of >1 million pigs (12). Since then, several NiV outbreaks have been recorded in Bangladesh and India (11). Fruit bats in the genus *Pteropus* (flying foxes) are the natural reservoir of both Hendra virus and NiV. NiV is present in fruit bat populations in Indonesia, Thailand, Malaysia, and Cambodia (9). In 1997, another new paramyxovirus, Menangle virus (MenPV), emerged as the cause of a disease outbreak in pigs causing stillbirth and abortion in a commercial piggery near Sydney, Australia (13). Two workers who were exposed to infected pigs developed a flu-like illness with rash and high titers of antibodies to MenPV (14). Seropositive flying foxes were found in a colony near the piggery, although MenPV was not isolated. Two years later, the fourth new paramyxovirus from bats, Tioman virus, was isolated from pteropid bat urine samples from Tioman Island off the east coast of Peninsular Malaysia (15). Tioman virus is related to MenPV, but its disease-causing

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Abbreviations: CPE, cytopathic effect; MDCK, Madin–Darby canine kidney; MelV, Melaka virus; MRV, mammalian orthoreoviruses; NBV, Nelson Bay orthoreovirus; NiV, Nipah virus; PulV, Pulau virus; RTI, respiratory tract illness; SARS, severe acute respiratory syndrome; $TCID_{50}$, tissue culture 50% infective dose.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession nos. EF026043–EF026046).

†To whom correspondence may be addressed. E-mail: chuakawbing@yahoo.com.sg or linfa.wang@csiro.au.

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status in animals and humans remains unknown. During the same period (1994–1999), Australian bat lyssavirus (ABLV) spilled over from bats to humans, resulting in two fatal infections (9, 16). Recently, we and another group independently identified horseshoe bats (genus *Rhinolophus*) as the reservoir host for a group of genetically diverse SARS-like coronaviruses (17, 18), suggesting that the etiological agent responsible for SARS had a bat origin. Although the exact origin of Ebola virus is still unknown, recent serological and molecular studies suggested a potential link between Ebola virus and bats (19).

The first reovirus of bat origin, NBV, was isolated in 1968 from the heart blood of a flying fox (*Pteropus poliocephalus*) in New South Wales, Australia (20). NBV was the first mammalian orthoreovirus to display the fusogenic properties characteristic of avian orthoreoviruses (21). In 1999, during a search for NiV in pteropid bats on Tioman Island, a novel orthoreovirus, Pulau virus (PulV), was isolated from *Pteropus hypomelanus* (22, 23). Serological and sequence characterization revealed that PulV was closely related to NBV. It is not known whether these bat orthoreoviruses are capable of infecting and causing disease in animals or humans.

Here, we report the discovery and characterization of Melaka virus (MelV), the third virus in the NBV species group, and its isolation from a human patient with fever and acute respiratory illness. Our data indicate that not only was the virus capable of human-to-human transmission, but also that its origin was probably bats. Furthermore, serological survey data suggest that the related bat-derived PulV is also capable of infecting humans, raising the possibility that PulV might have caused respiratory illness that was undiagnosed or misdiagnosed in the past.

Results

Virus Isolation from a Patient with Acute Respiratory Disease. On March 20, 2006, a 39-year-old male patient (MRA) with a 1-day history of high fever, cough, and sore throat was treated in a government outpatient clinic in the suburb of Melaka, the capital city of the state of Melaka, in Malaysia. His high-grade fever was associated with headache, myalgia, malaise, loss of appetite, generalized body weakness, and severe prostration. There was no associated giddiness, blurring of vision, photophobia, skin eruption, conjunctivitis, or arthritis or related episode of abdominal pain, nausea, vomiting, or diarrhea. The high fever and associated symptoms persisted for 4 days. The patient was treated by different doctors on three occasions during his illness. His cough was initially mild and associated with coryza. Over the subsequent week, his cough worsened progressively with production of yellowish mucoid sputum. However, there was no associated dyspnea, tachypnea, or hemoptysis, although there was one episode of epistaxis. His sore throat, described as severe in nature, persisted for 4 days and was associated with a sensation of tightness in the pharynx and neck, which led to some degree of difficulty in swallowing both liquid and solid food. He continued to feel weak and lethargic for nearly a fortnight after defervescence.

Physical findings at first examination showed MRA to be a Malay man of medium build who appeared unwell and tired. His oral temperature was 39°C and vital signs were stable, with a recorded blood pressure of 120/80 mmHg (1 mmHg = 133 Pa) and pulse rate of 80 beats per min. He was not cyanosed, jaundiced, or in respiratory distress. There was no cutaneous rash or petechiae. His throat displayed injection and hyperemia with slight enlargement of the tonsils but was free of white exudates. Air-entry into his lungs was equal and breath sound was bronchovesicular without other adventitious sounds. The abdominal examination was normal and hepatosplenomegaly was not noted. A mobile, slightly tender cervical lymph node (≈1 × 1.5 cm) was palpable in each anterior triangle of his neck. Other systemic examinations were essentially normal, and a provisional clinical diagnosis of influenza-like illness was made

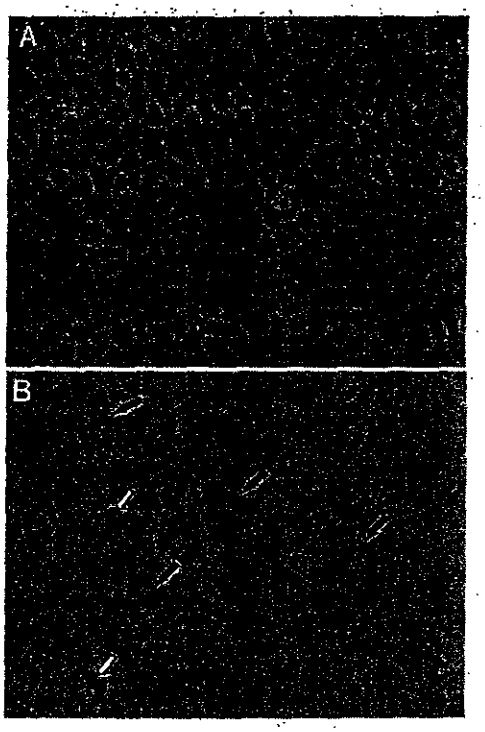

Fig. 1. Syncytia formation in MelV-infected MDCK cells. (*A*) Mock-infected. (*B*) MelV-infected. Two types of syncytial cells were observed: cells still attached to culture flask surface (filled arrows) and cells detaching from the surface (open arrows).

at first examination. Neither chest x-ray nor venous blood was taken, but a throat swab was collected and transported in viral transport medium to a laboratory for virus isolation.

A virus causing a syncytial cytopathic effect (CPE) in Madin–Darby canine kidney (MDCK) cells (Fig. 1) was isolated after 48 h incubation at 37°C. The virus was named "Melaka virus" (MelV) after the location of the index case. After two passages in MDCK cells, MelV replicated and caused syncytial CPE in all types of mammalian cell lines available in the laboratory and also in mosquito-derived C6/36 cells [see supporting information (SI) Table 2].

MelV-infected cells failed to react to antiserum against most known respiratory viruses, including adenovirus, influenza A and B, parainfluenza 1, 2, and 3, and respiratory syncytial virus. PCR analyses of the original throat swab by using primers against these viruses were also negative (data not shown).

Morphological and Serological Characterization. Transmission electron microscopical examination of ultrathin sections from MDCK and Vero cells exhibiting a syncytial CPE and associated negative-stained particles from the supernatant revealed icosahedral viruses resembling those of the family *Reoviridae*, genus *Orthoreovirus*. The particles possessed a mean diameter of 74.20 ± 5.02 nm (n = 43) in negative-stained preparations and 78.86 ± 5.31 nm (n = 37) for individual viruses in ultrathin sections. The viruses were naked and possessed a double capsid with conspicuous "spikes" or "turrets" situated on the inner core. Within ultrathin sections, viruses were observed in the cytoplasm as individual particles in paracrystalline arrays and associated with semi-electron-dense inclusion bodies (Fig. 2).

The growth characteristics of MelV and PulV were similar in a number of cell lines, although subtle differences existed in the degree of syncytial CPE that each virus induced (SI Table 2).

Results obtained from cross-neutralization studies (SI Table 3)

Fig. 2. Electron micrographs of MelV. (*A*) Negative-stained MelV. (Scale bar: 200 nm.) (*B*) Image of an ultrathin section of a MelV-infected multinucleated Vero cell. N, nucleus; arrows, paracrystalline viral arrays (inclusion bodies not shown). (Scale bar: 5 μm.) (*C*) Higher magnification view of a paracrystalline array. (Scale bar: 200 nm.)

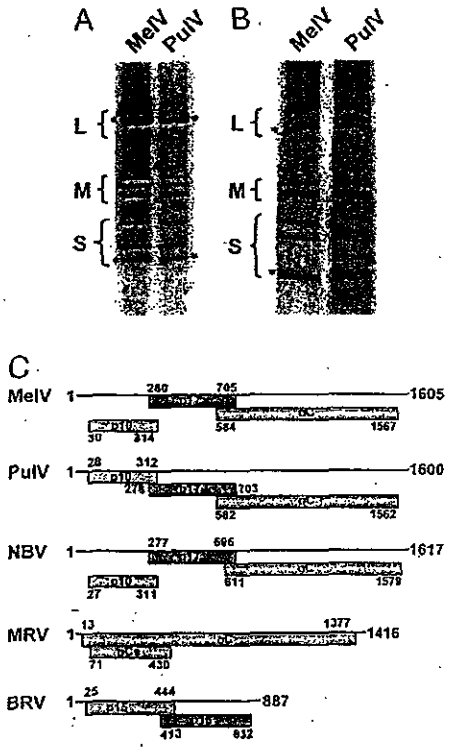

Fig. 3. Comparison of genome segments and coding strategy of the polycistronic S-class genome segments. (*A*) Genome segments of MelV and PulV separated on a 1% agarose gel. (*B*) Genome segments of MelV and PulV separated on a 10% SDS-polyacrylamide gel. The classes of genome segments (L, large; M, medium; S, small) are labeled on the left, and the asterisks indicate comigrating bands where more than one segment is present. (*C*) Coding arrangement of the polycistronic S segments of three bat orthoreoviruses in comparison with two other mammalian orthoreovirus species. The line on top represents the RNA genome and the shaded boxes underneath depicture protein-coding regions in reading frames 1–3 (top to bottom). Numbers refer to size, in base pairs, of the genome segments and to the first and last nucleotides of the individual ORFs (excluding the stop codons). The names of the encoded proteins are indicated within the shaded boxes.

indicated that serum from the patient (MRA) neutralized both MelV and PulV with equal efficacy. Similarly, rabbit anti-PulV serum cross-neutralized MelV but not any of the three MRV types. Vice versa, none of the three MRV-type sera used in this study was able to neutralize either MelV or PulV. All members of the family appeared to have preexisting anti-MRV2 antibodies that showed no correlation with their anti-MelV antibody status.

Molecular and Phylogenetic Characterization. Comparison of genome segments by gel electrophoresis revealed almost identical electropherotypes for MelV and PulV (Fig. 3*A* and *B*). Sequence analysis of the four small (S) segments indicated a very close genetic relationship between the two viruses. One S class genome segment of orthoreoviruses may be polycistronic, and its coding arrangement is characteristic of a particular species group (24). As shown in Fig. 3*C*, the S1 segment of MelV has an almost identical coding arrangement to that of PulV and NBV. Another genetic marker often used for species differentiation of orthoreoviruses is the highly conserved genome terminal sequences at the 5′ end of the positive-sense RNA (7). For MelV, the consensus sequence is 5′-GCUUwA (w = U or A), which is identical to that of PulV and NBV (SI Fig. 5). The deduced protein products encoded by the four S segments of the two viruses are very similar in size and share high levels of sequence identity (SI Figs. 6–9).

To establish the evolutionary relationship among MelV, PulV, and other known orthoreoviruses, phylogenetic trees were constructed based on deduced amino acid sequences of the major outer capsid protein (Fig. 4*A*) and the major inner capsid protein (Fig. 4*B*), respectively. In both phylogenetic trees, MelV was most closely related to PulV and was placed in the species group III together with NBV. Similar trees were obtained based on other proteins encoded by the S genome segments (data not shown).

Epidemiological Investigation. MRA presently holds the rank of sergeant in the Malaysian Army and works as an accounts clerk in a military base in Melaka. He has not been involved in jungle training or overseas field trips for the last 2 years. However, an unusual event occurred ≈1 week before the onset of his fever. On that day, a bat flew through the main door into his living room at ≈8 p.m. while he was watching television. The bat flew "frantically" for ≈2–3 min in the living room without making any "squeaky" noise or alighting on the ceiling and subsequently managed to fly out through the same door through which it entered.

MRA is married with five children aged 12, 11, 8, 6, and 2, and his wife was 8 months pregnant at the time of his illness. Approximately 6 days after the onset of his fever, his second

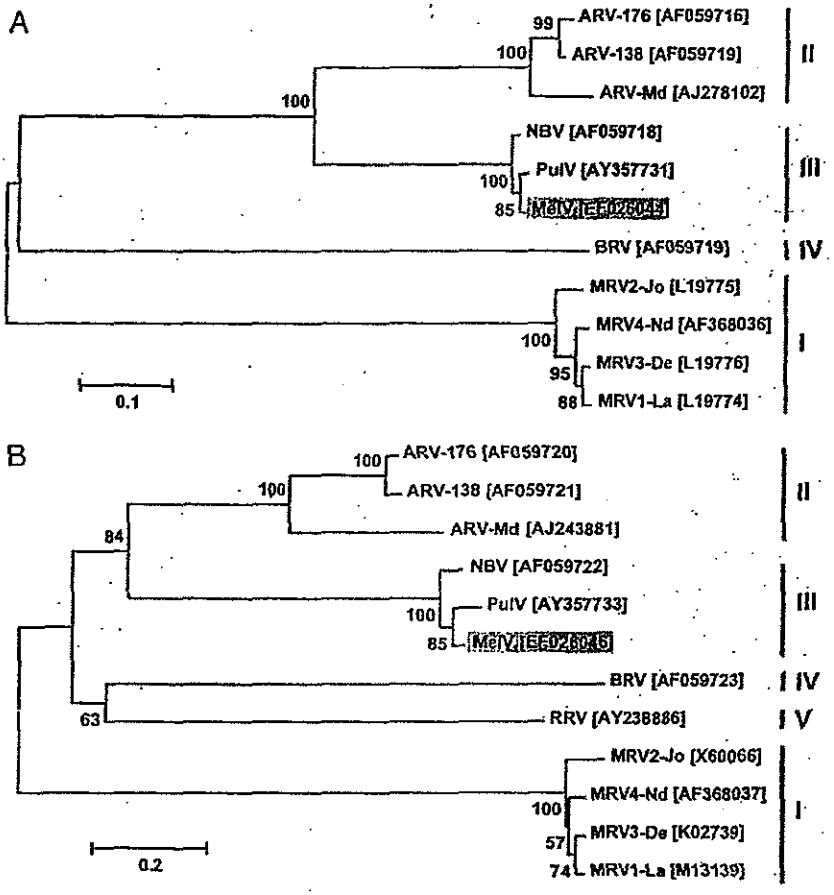

Fig. 4. Phylogenetic trees of orthoreoviruses based on deduced amino acid sequence of the major inner (*A*) and outer (*B*) capsid proteins. The GenBank accession number for each sequence is given in bracket next to the abbreviated virus name. ARV, avian reovirus; BRV, baboon reovirus; RRV, reptilian reovirus. Numbers at nodes indicate levels of bootstrap support calculated from 1,000 trees.

child (his 11-year-old daughter) developed a high fever; his fourth child (his 6-year-old son) developed a high fever the next day. Both children were lethargic for 1 day but did not have a cough, sore throat, or skin rashes, although the fourth child had one episode of vomiting. His wife did not experience any illness or subjective feeling of being unwell. She gave birth to a normal, healthy baby girl on April 16, 2006.

MRA and his family live in a single-story linked house in a residential area of 30 houses situated in the suburb of Melaka. The family did not keep any pets or rear any poultry in the house compound, although a neighbor who lived five houses away kept a few parrots.

On follow-up investigation, venous blood samples were taken from MRA on three separate occasions. Venous blood samples were also taken from his family members on the third occasion. The sera from the patient and his family members were tested for the presence of antibodies against MelV, and the results are summarized in Table 1. Similar results were also obtained when the sera of the different family members were examined by immunogold electron microscopy. Whereas the sera of MRA, his wife, his 11-year-old daughter, and 6-year-old son were able to specifically react to MelV, the sera from his other sons were negative (data not shown).

Seroprevalence. To test the prevalence of MelV or other closely related reoviruses in the human population, we used a panel of human sera collected in 2001–2002 from volunteers on Tioman Island during serological surveillance for NiV (25). This panel of sera was chosen because a large number of fruit bats were present on the island and several isolates of PulV were made from bat urine specimens collected on the island at the time. The sera were tested against both PulV and MelV. The data obtained indicate that ≈13% of the 109 sera tested neutralized both viruses. Some sera neutralized both viruses equally well, but several showed a slightly higher neutralizing activity against PulV than MelV (see SI Table 4).

Discussion

The last decade has experienced a surge in the discovery of emerging viruses of bat origin, several of which have had significant impact on human and animal health, tourism, and trade (26–29). Isolation of MelV from a human patient and demonstration of human infection by the closely related PulV serve as the latest examples of this emerging trend. Although we cannot discount the role of modern detection technology in facilitating identification of new viruses, it is believed that ecological changes, which result in greater interaction of human and wildlife animals, are mainly responsible for the increased spillover events observed in recent years (26–29).

A number of viruses recently emerged from bats display a "promiscuous" host range. Such viruses may have coevolved with bats, which are considered to be among the most ancient mammalian species, and use cell-surface receptors that are conserved across a range of animal species. This is certainly the case for henipaviruses, which naturally infect >10 different species in six mammalian taxonomic orders, an unusual obser-

Table 1. Serological responses of the patient and his family members to MelV

Person (age)	First bleed (April 3)	Second bleed (May 12)	Third bleed (June 9)
Immunofluorescence antibody tests titer IgM/IgG			
MRA (39)	1:80/1:640	1:40/1:2,560	1:10/1:2,560
Wife (36)	NA	NA	1:20/1:2,560
Son (12)	NA	NA	−ve/−ve
Daughter (11)	NA	NA	1:10/1:320
Son (8)	NA	NA	−ve/−ve
Son (6)	NA	NA	1:10/1:80
Son (2)	NA	NA	−ve/−ve
Virus neutralization test titer			
MRA (39)	1:80	1:320	1:160
Wife (36)	NA	NA	1:40
Son (12)	NA	NA	−ve
Daughter (11)	NA	NA	1:160
Son (8)	NA	NA	−ve
Son (6)	NA	NA	1:40
Son (2)	NA	NA	−ve

NA, sera not available for testing; −ve, negative.

vation for paramyxoviruses whose host ranges are usually limited (11). Ephrin B2 has been identified as the main receptor for henipaviruses (30, 31), and its wide distribution and status as a member of a conserved family of cell-surface glycoprotein ligands (32) may help to account for the wide host range of henipaviruses *in vivo* and *in vitro*. Similarly, the receptor for the SARS coronavirus, angiotensin-converting enzyme 2 (33), is also conserved among different animals (34), an observation consistent with the finding that SARS coronavirus seems to be able to infect most mammalian species tested under experimental conditions (35). In this regard, it is worth noting that MelV and PulV are also capable of replicating in a wide range of cell lines, including one derived from mosquitoes (see SI Table 2).

The risk of virus spillover from bats as a result of increasing encroachment by animals and humans into bat habitats is enhanced by the genetic diversity observed among newly emergent bat-borne viruses. For henipaviruses, four to five genetically distinct virus strains have been detected with different virological and biological properties. For example, the NiV Bangladesh strains, but not the NiV Malaysian strains, are capable of human-to-human transmission (11). This is also true for the SARS-like coronaviruses in bats. A limited search among horseshoe bats in China already uncovered at least five different genetic variants (17, 18), and the number of variants is expected to increase dramatically when a systematic survey is conducted across different countries and geographic areas. The same applies to the NBV species group, which has three different members so far. Considering the wide distribution of pteropid bats in India, Southeast Asia, Australia, and the Pacific, one can expect that more bat-borne reoviruses in this group will be discovered in the future. It should be noted that although the epidemiological tracing data and the close genetic relationship of MelV to PulV and NBV strongly suggest that MelV originated from bats, at this stage we do not have experimental evidence to prove the bat origin of MelV and the direct bat-to-human transmission. Field surveillance of bat populations in Melaka will be necessary to resolve this issue.

MelV is a unique orthoreovirus capable of infecting and causing disease in humans. The infection of multiple members in the same family and the delayed onset (by 1 week) of clinical symptoms of the two children strongly suggest human-to-human transmission. It is not clear to us how and when the wife became infected and why she displayed no clinical symptoms. Fusogenicity is a known important virulence marker for many of the well characterized respiratory viruses, such as respiratory syncytial virus, henipaviruses, influenza, and parainfluenza viruses. Although widely present among human populations, none of the MRVs are fusogenic, an observation highlighting the potential importance of cell–cell fusion for the pathogenesis of orthoreoviruses in humans.

In a recent Foresight (www.foresight.gov.uk) report entitled "Infectious Diseases: Preparing for the Future," eight categories of infectious diseases were identified for which improved detection systems would make a difference over the next 10–25 years (36). Of the eight categories, MelV represents categories one, three, and six: new diseases, zoonoses, and acute respiratory infections. With the advent of multiplexed diagnostic technologies both at the nucleic acid and protein levels, classic exclusion diagnosis will eventually be replaced with differential diagnosis. When a severe RTI patient presents at a hospital, it is important not only to exclude SARS or highly pathogenic avian influenza, but also to accurately determine the causative agent so that a targeted treatment regimen can be implemented. In this regard, identification of novel RTI agents will play a key role in shaping the future of RTI diagnosis, and the discovery of MelV, its relationship with PulV and NBV, and the potential involvement of fusogenic reoviruses as causes of RTI will undoubtedly make a significant contribution to the improvement of RTI diagnosis and treatment.

Materials and Methods

Virus Isolation and Purification. The throat swab sample in viral transport medium was treated with antibiotics (100,000 units/ml penicillin and 100 μg/ml streptomycin). After 1 h of treatment, the sample was inoculated in duplicates (100 μl and 200 μl, respectively) into freshly confluent monolayers of MDCK (ATCC, CCL-34), Vero (ATCC, CCL-81), and Hep-2 (ATCC, CCL-23) cells cultured in a 24-well cell culture plate. The plate was incubated at 37°C in 5% CO_2 and examined daily for the presence CPE in cultured cells. After two passages in MDCK, the virus (MelV) was passaged once in Vero cells in the presence of ciprofloxacin (10 μg/ml) to control mycoplasma contamination. The virus was purified by three consecutive limiting dilution passages in Vero cells in the presence of ciprofloxacin. After purification, the virus was passed twice at low multiplicity of infection in Vero cells and harvested 72 h postinfection to generate a working stock for analysis. Stock preparations of MelV had average titers of 4×10^6 $TCID_{50}$ (tissue culture 50% infective dose)/ml. PulV was previously plaque-purified (23) and grown in Vero cells to a titer of 1×10^6 $TCID_{50}$/ml. Virus preparations were purified for further analysis according to standard protocols by using Dounce homogenization and ultracentrifugation.

Electron Microscopy. Vero cells displaying syncytial CPE were processed through to resin blocks and ultrathin sections examined at 120 kV in a Philips CM120 transmission electron microscope (Philips, Eindhoven, The Netherlands). For negative staining, paraformaldehyde-fixed (2%) purified MelV and stocks of PulV were adsorbed onto carbon-coated parlodion-filmed nickel grids (5 min), stained with 2% (wt/vol) phosphotungstic acid (pH 6.8) (1 min), and viewed as described above.

Antibody Tests. Immunofluorescence antibody tests (IFAT) were conducted as follows. A freshly confluent monolayer of MDCK was inoculated with MelV at high multiplicity of infection and incubated at 37°C in 5% CO_2. At full CPE, the infected cells were harvested, washed four times, and suspended in sterile PBS at a cell concentration of 3,000 cells per ml. Ten microliters of the infected cell suspension was carefully spotted onto each well of Teflon-coated slides, followed by air-drying over a warm plate and subsequent fixation in cold acetone for 10 min. Five micro-

liters of each serum was subjected to serial 2-fold dilution with sterile PBS from 1:10 to 1:2,560. For assay of IgM, IgG was removed by absorption with protein A before serum dilution. Twenty microliters of diluted serum was transferred onto each respective well of the antigen-coated slide and incubated in a moist chamber for 30 min at 37°C. Subsequently, the slides were rinsed with PBS before being soaked for 10 min in PBS solution that was kept in gentle motion by a magnetic stirrer. The slides were allowed to air-dry over a warm plate and probed with 20 μl of 1:40 diluted fluorescein-conjugated respective rabbit anti-human IgM or IgG (Dako, Glostrup, Denmark). The slides were then incubated for another 30 min at 37°C in a moist chamber. The same process of washing and drying was carried before the slides were mounted with a commercially supplied mounting fluid, and the specific reactivity/labeling was read under a UV fluorescence microscope (BX50; Olympus, Tokyo, Japan) at ×400 magnification.

For the virus neutralization test of PulV or MelV, serial 2-fold dilutions of control and test sera were prepared in duplicate starting at 1:10. An equal volume of either MelV or PulV working stock containing 150 $TCID_{50}$ was added to the diluted sera and incubated for 30 min. The preincubated virus/serum mix was added to confluent Vero cell monolayers and incubated for 1 h. The inoculum was removed, monolayers were washed three times with PBS, and cell media were replaced. All incubations were performed at 37°C in a humidified 5% CO_2 incubator. Vero cell monolayers were observed for CPE 3 days later. The ability of sera to neutralize virus was determined by scoring the extent of CPE observed in duplicate wells.

For the virus neutralization test of MRV, MA104 cell monolayers were prepared in 96-well tissue culture plates seeded with 20,000 cells per well and incubated overnight at 37°C in a humidified 5% CO_2 incubator. MRV types 1, 2, and 3 were diluted in cell media to give 4×10^4 TCID50/ml, and 100 μl of virus was added to wells in a 96-well incubation plate. Serial 10-fold dilution of test sera were prepared starting from 1:10, and 100-μl aliquots were added to virus wells. The sera/virus mixture was incubated for 60 min at 37°C. The medium was discarded from the cell monolayers, and four 50-μl replicates of the preincubated virus/sera mix were transferred from the incubation plate to appropriate wells in the test plate. At the same time, 12 virus control wells were prepared by adding 10^3 $TCID_{50}$ per well from the above diluted virus solution, and the plates were incubated for 30 min at 37°C. The inoculum was discarded and the cells were washed three times with 200 μl of cold PBS, followed by the addition of 100 μl of fresh media. After incubation at 37°C for 20 h, the medium was discarded, the cells were fixed in 100% ice-cold methanol for 15 min and air-dried, and the wells were blocked with 200 μl of 1% BSA/PBS. The neutralization of MRV was monitored by immunofluorescent microscopy with rabbit anti-MRV antibodies and goat anti-rabbit conjugated with Alexa Fluor 488 (Invitrogen, Carlsbad, CA). Fluorescence was observed by using an Olympus fluorescent microscope as described above.

Sequence and Phylogenetic Analysis. Extraction and purification of dsRNA and synthesis of randomly primed cDNA were carried out as described (23, 37). Primers (primer sequences will be supplied upon request) designed by using PulV and NBV small genome segment sequences were used for PCR amplification and sequencing of the MelV small genome segments. Genome segment terminal sequences were obtained by using a two-step PCR amplification procedure, first by the single primer amplification technique (SPAT) (37), then by a seminested PCR using the combination of MelV genome segment-specific primers and the adaptor-specific primer used in the single primer amplification technique. Phylogenetic trees were constructed by using the neighbor-joining algorithm with bootstrap values determined by 1,000 replicates in the MEGA3 software package (38).

Serological Survey. The panel of human sera collected during an investigation of the potential risk of bat-to-human transmission of NiV (25) was used in this study. The original panel consisted of 153 serum samples collected from adult residents (mean age 38 ± 15 years) of Tioman Island, which represented 8% of the total adult population on the island. Due to the supply shortage and poor quality of some serum samples, only 109 sera were tested in this study. The sera were heat-inactivated by incubation at 56°C for 30 min before being tested for MelV- or PulV-specific antibodies by using the virus-neutralization test described above.

We thank C. T. Tan, V. H. T. Chong, K. T. Wong, and K. J. Goh for their permission to use the panel of human sera collected from Tioman Island; G. Smith for providing the MRV prototype viruses; K. McPhie for providing MRV sera; K. Selleck for technical assistance; and T. Pye and E. Hansson for help with DNA sequencing.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
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一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ProMED 20070702-2108, 2007 Jul 2. 情報源:Martin Bel, Chairman of the Yap State EpiNet Team, Yap State Department of Health Services, Federated States of Micronesia.	公表国 ミクロネシア
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研究報告の概要

○ジカウイルスのアウトブレイク―ミクロネシア(ヤップ島)確定
2007年6月22日、ヤップ州保健省が収集した血液検体を米国CDC(疾病対策予防センター)の研究所で検査した結果、ヤップ島の感染症はジカウイルスによるものと考えられた。ヤップのアウトブレイクは2007年4月に始まり、5月の後半にピークを迎えたものと見られる。新規症例の発生は続いている。症状は、時に掻痒感を伴う斑点状丘疹、結膜炎、関節痛など軽症で、発症期間は通常4～7日間である。
6月29日までにPCRとIgM検査でジカウイルス感染が確認された症例は42例になった。さらに、上記の症状のうち少なくとも2つを発症した65例の可能性例が、ヤップ記念病院及びWa'abの保健所を受診した。入院、死亡した患者はいない。症状が軽いため、受診していない患者も多いと考えられる。この地域における初期の分析では、相当数の人が感染していることが示された。ヤップ島全土で症例が発生している。ジカウイルス感染患者は、ヤップで使用されているデングの迅速検査(PanBio及びPentax)で偽陽性反応を示す。伝統的にジカウイルスによる疾患は「ジカ熱」と呼ばれてきたが、今回のアウトブレイクでは発熱は見られない。ヤップのアウトブレイクで見られた他の症状は、眼窩後部痛、筋肉痛、下肢の浮腫、リンパ節症、下痢である。
ヤップでは蚊の産卵場所を削減するためのキャンペーンを強化している。ヤップ州政府は、長袖の服や防虫剤の使用などで蚊に刺されないよう呼びかけている。同保健省は、ミクロネシア連邦政府、WHO、パスツール研究所、米国CDCと協力して、疾患の臨床症状、アウトブレイクの規模、伝播様式を特定し、最良の予防法を決定するためにさらなる調査を行っている。

使用上の注意記載状況・その他参考事項等

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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
ミクロネシア連邦ヤップ島で流行した感染症がジカウイルスによるものと考えられたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。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。

Archive Number 20070702.2108
Published Date 02-JUL-2007
Subject PRO/AH/EDR> Zika virus outbreak - Micronesia (Yap) (02): confirmed

ZIKA VIRUS OUTBREAK - MICRONESIA (YAP) (02): CONFIRMED
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A ProMED-mail post
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International Society for Infectious Diseases
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[1] Micronesia (Yap)
[2] Africa

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[1] Micronesia (Yap)
Date: Fri 29 Jun 2007
Source: Martin Bel, Chairman of the Yap State EpiNet Team, Yap State Department of Health Services, Federated States of Micronesia <mbel@fsmhealth.fm>

On 22 Jun 2007, blood specimens collected by the Yap Department of Health Services and tested at CDC (Centers for Disease Control) labs showed that the recent illness in Yap is likely caused by the Zika virus.

The Yap outbreak appears to have started in April 2007 and to have peaked in late May [2007]; however, cases continue to present. In this outbreak the symptoms are mild and generally last for 4-7 days, consisting of:
* a maculopapular rash involving the trunk and extremities that is sometimes pruritic;
* conjunctivitis; and
* joint pain that can affect both large joint and the smaller joints of the hands and feet.

As of Thu 29 Jun [2007], there have been 42 cases confirmed to be Zika by PCR [polymerase chain reaction] and IgM analysis. An additional 65 probable cases with at least 2 of these symptoms have presented to the outpatient department of the Yap Memorial Hospital and at the Wa'ab Community Health Center. No patients have been admitted to the hospital and there have been no deaths. Because the disease is mild, many more infections are thought to have occurred in the community that did not seek medical attention. An initial assessment in the community indicates that a significant proportion of the population has been affected. Geographically, cases have occurred all over the island of Yap.

Rapid tests used in Yap for dengue have given false positive results on patients with Zika virus (PanBio and Pentax).

Traditionally, illness caused by Zika was termed "Zika Fever." However fever has been an inconsistent feature of this Zika outbreak. Other symptoms that have been noted in the Yap outbreak include retro-orbital eye pain, myalgias, lower extremity edema, lymphadenopathy, and diarrhea.

An enhanced environmental campaign to reduce mosquito-breeding areas in Yap is ongoing. The Yap community has been asked to avoid mosquito bites by wearing long clothing, staying within screened areas and using mosquito repellents and coils, especially when ill.

The Yap Department of Health Services is conducting further investigations to better characterize the clinical presentation of the illness, the magnitude of the outbreak, and the mode of transmission in order to determine the best control measures. They will be continuing to work with FSM [Federated States of Micronesia] Department of Health, Education, and Social Services, the WHO, Institut Pasteur, and the CDC.

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Communicated by:
Dr. Steve Berger <mberger@post.tau.ac.il>
Dr. Charles H Calisher <calisher@cybercell.net>

[The Zika virus etiology in this epidemic appears to be clear, given the PCR results, although this report does not mention isolation of the virus. ProMED-mail awaits with interest further results of the investigations that the Yap Department of Health Services plans to carry out.

A map of Micronesia can be accessed at <http://www.lib.utexas.edu/maps/islands_oceans_poles/micronesia_pol99.jpg>. - Mod.TY]

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[2] Africa
Date: Fri 29 Jun 2007
From: Bernard Mondet <bernard.mondet@ifpindia.org>

The Institut Pasteur / IRD (Institute for Research and Development) database on viruses in Africa mentions 23 species of mosquitoes harboring Zika virus from West Africa countries and provides several references. Please, look at:
<http://www.pasteur.fr/recherche/banques/CRORA/virus/v010180.htm> (in French)

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Bernard Mondet
Institut Francais de Pondicherry
CP 33 11, Saint-Louis Street
Pondicherry, 605-001, India
<bernard.mondet@ifpindia.org>

[ProMED-mail thanks Dr. Mondet for these references, citing the isolation of Zika virus from mosquitoes in Senegal, Cote d' Ivoire, the Central African Republic, and Burkina Faso. Most of the isolates are from various _Aedes_ spp, _Eremapodites_ spp., and _Mansonia uniformis_.
This site also lists several references that cover field and experimental work (articles mainly in French). - Mod.TY]

[see also:
Zika virus outbreak - Micronesia (Yap): susp, RFI 20070627.2065]
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医薬品
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識別番号・報告回数			報告日	第一報入手日 2007 年 7 月 27 日	新医薬品等の区分	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン		研究報告の公表状況	The New York Times/July 26, 2007	公表国 アメリカ	
販売名 （企業名）	①献血ヴェノグロブリン-IH ヨシトミ（ベネシス） ②ヴェノグロブリン-IH（ベネシス） ③グロブリン-Wf（ベネシス）					

研究報告の概要

米国における WNV 症例の数は 1 年前の数のおよそ 4 倍であり、大きな流行が控えている可能性があることを意味していると、政府研究者が報告している。
昨年は、米国で 4,269 の症例が報告されが、この中には 1,495 の脳症が含まれ、177 人が亡くなっている。
今年はこれまで 122 症例が報告され、カリフォルニア州と南北ダコタ州で最も多かった。今年は既に、42 の脳症と 3 件の死亡があった。一方、昨年のこの時期は、報告はわずか 33 症例であった。
過去の症例では、このウイルスは、輸血及び臓器移植によって伝播したが、今は血液供給を保護するために検査が実施されている。今年は、この検査によって、感染している可能性のある 23 の血液ドナーが明らかになった。

報告企業の意見

今年の WNV 感染者数が昨年同時期に比べておよそ 4 倍であり、大きな流行になる可能性があるとの報告である。FDAは、2005年6月の業界向けガイダンス改訂版において、「FDAは全ての血漿分画製剤について現在行われているウイルス低減工程を再調査した。現在行われている方法は、WNVと分類上関連しているフラビウイルスを不活化することがバリデートされている。」と評価し、CPMPもまたポジションステートメントにおいて、血漿分画製剤の製造工程でWNVは不活化・除去されると評価している。万一、原料血漿にWNVが混入しても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。

今後の対応

本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）及び pH 3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

July 26, 2007

Rise in Cases of West Nile May Portend an Epidemic

By DENISE GRADY

The number of West Nile virus cases in the United States is nearly four times what it was a year ago, meaning that a large epidemic may be in store, government researchers are reporting.

"It's certainly a warning sign that we need to be extremely vigilant," Dr. Lyle Petersen, the director of the division of vector-borne infections at the Centers for Disease Control and Prevention, said yesterday. "The worst is yet to come."

The virus, carried by mosquitoes, causes a mild, flu-like illness in 20 percent of those infected, and no symptoms in about 80 percent. In about 1 percent of cases, the illness progresses to a brain infection that can be fatal.

Last year, 4,269 cases were reported in the United States, including 1,495 brain infections, and 177 people died. The risk of severe illnesses increases with age.

So far this year, 122 cases have been reported, with the most in California and the Dakotas. At this time last year, there had been only 33.

The reported cases are just the tip of the iceberg, researchers say. Many infections are never diagnosed because they were mild and the patient did not see a doctor, or was not tested for the virus.

This year, there have already been 42 brain infections and 3 deaths. This is early in the season, since 90 percent of the cases usually occur in August and early September. It is impossible to predict whether the trend will continue, Dr. Petersen said, adding that it may be related to "a lot of weird weather events," including both the heat waves in the West and unusual storm patterns in the Midwest.

If people keep getting infected at the current rate, he said, "we could see the largest epidemic ever."

The first known case of the disease in the United States occurred in New York City in 1999, and since then the virus has spread to every state.

In cases in the past, the virus was transmitted by transfusions and organ transplants, but tests are now done to protect the blood supply. This year, the tests have found 23 potential blood donors who were infected.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 8. 31	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)		金平克史, 白藤浩明, 神尾次彦. 第144回日本獣医学会学術集会(抄録集); 2007 Sep 2-4; 江別市.	日本

研究報告の概要

○日本産蚊のウエストナイルウイルス増殖・媒介能

ウエストナイルウイルス(WNV)は野鳥から蚊によって人や馬に伝播しウエストナイル熱や脳炎を引き起こす。WNV 感染症はヨーロッパ諸国、極東地域をふくむロシアでは散発的に発生があり、近年、北米への感染域拡大がおこった。近い将来、日本にも侵入する可能性があるため、日本産蚊の室内継代株を用いて本ウイルスの感受性を検討した。アカイエカ、ヒトスジシマカ2系統、オオクロヤブカの雌にWNV NY99株を10^3 PFU／蚊となるように腹部に注入した(注入群)。また、アカイエカ、ヒトスジシマカに感染ジュウシマツを吸血させた(吸血群)。これらの蚊を湿度70～80%、室温25℃で維持し、7日目および14日目に各群の蚊10匹を用いてウイルス力価を測定した。加えて14日目に各群10匹の蚊あたり1匹のマウスを吸血させ媒介試験を行った。媒介試験は各群3回行った。注入群、吸血群ともに全種類の蚊においてウイルスの増殖が観察された。全ての蚊が7日目で10^4 PFU／蚊以上、14日目で10^6 PFU／蚊以上のウイルス力価を示した。媒介試験では、アカイエカ注入、吸血両群、ヒトスジシマカ2系統の注入群、1系統の吸血群では供試したすべてのマウスが8日以内に立毛や後躯麻痺の症状を示し、12日以内に死亡した。死亡したマウスからはWNV が検出された。ヒトスジシマカ1系統の吸血群とオオクロヤブカ注入群では2匹のマウスが同様の経過を示した。感染ジュウシマツを吸血したアカイエカとヒトスジシマカがマウスにウイルスを媒介したことから、野外においてこれら日本産蚊が鳥類から哺乳類への媒介を担う可能性が示された。

報告企業の意見	今後の対応
感染実験により、日本産蚊が鳥類から哺乳類へウエストナイルウイルスの媒介を担う可能性が示されたとの報告である。	輸血によるWNV感染リスクを防止するため、国の指示(平成16年7月13日付薬食発第0713008号「ウエストナイルウイルス等の輸入感染症対策に係る採血禁止期間の変更について」)により帰国(入国)後4週間の献血を禁止している。また、WNV感染の発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき、緊急対応の準備を進めている。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

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DV-49 豚コレラウイルス感染細胞内における自然免疫関連因子 Interferon Promoter Stimulator-1 (IPS-1) の動態

○野村拓志[1]、迫田義博[1]、松野啓太[1,2]、喜田宏[1,2]（[1]北海道大学・獣医学部微生物学教室、[2]北海道大学・人獣共通感染症リサーチセンター）

【目的】豚コレラウイルス(CSFV)感染細胞ではウイルス非構造蛋白質 N^{pro} がプロテアソーム系を介してインターフェロン(IFN)産生調節因子-3(IRF-3)の発現量を減少させ、自然免疫に関与する1型IFN産生を抑制している(Hiltonら、2006)。一方、同じフラビウイルス科に属するC型肝炎ウイルス(HCV)の感染ではウイルス非構造蛋白質が細胞のミトコンドリア膜上蛋白質である Interferon Promoter Stimulator-1(IPS-1)を分解不活化し、1型IFN産生を抑制することが示唆されている(Johnsonら、2007)。本研究はCSFV感染細胞における1型IFN産生にIPS-1がどのように関与するかを明らかにするために企図された。【材料と方法】ウシ、ヒト、マウス由来のIPS-1遺伝子の塩基配列よりプライマーを作製し、豚腎株化細胞(CPK細胞)からブタIPS-1の遺伝子をクローニングした。大腸菌でブタIPS-1を組換え蛋白として調製し、これに対する特異抗体を作出した。本抗体を用いてウイルス感染細胞中のIPS-1の発現量と局在をウエスタンブロット法および蛍光抗体法により解析した。【結果】強毒株であるALD/A76感染細胞ではIPS-1の発現量が経時的に増加した。一方弱毒生ワクチン株であるGPE-感染細胞でもIPS-1発現量が経時的に増加し、その発現量はALD/A76株のそれよりも高かった。CSFV感染細胞と非感染細胞でIPS-1の細胞内局在性に相違は認められなかった。【総括】以上の結果からCSFV感染細胞ではIPS-1発現量が増加し、その程度はウイルス株により異なることが明らかとなった。また、CSFVの感染によってIPS-1が分解されないことが明らかとなった。また、この知見はHCV感染で観察された結果と異なるものである。現在これらの現象の分子基盤を解析している。

DV-50 牛ウイルス性下痢ウイルス持続感染牛におけるウイルスRNA蓄積レベルと先天性免疫誘導の関係

○山根大典[1]、加藤健太郎[1]、遠矢幸伸[1]、明石博臣[1]（[1]東京大学・獣医微生物学研究室）

【背景と目的】牛ウイルス性下痢ウイルス(BVDV)は非細胞病原性(ncp)株が胎子に経胎盤感染し、免疫寛容となった子牛に持続感染することで下痢や削痩の他、免疫抑制に伴う病態を示し、やがて細胞病原性(cp)株の重感染により致死的な粘膜病を発症すると考えられている。我々はこれまでBVDV感染時に誘導される宿主因子の解析を *in vitro* で行ってきたが、その知見を元に持続感染(PI)牛の病態形成に関与する宿主免疫応答を探索した。

【方法と材料】我々はcp株感染培養細胞において2本鎖RNA依存性プロテインキナーゼ(PKR)、オリゴアデニレートシンセターゼ1(OAS-1)、TNFalpha、iNOSがウイルスRNAの蓄積により誘導されることが細胞病原性発現に関与していることを報告した（第53回日本ウイルス学会）。この *in vitro* での成績を元に、今回PI牛と非感染牛の臓器中の宿主因子の転写量及びウイルスRNA量をリアルタイムPCRにより定量解析した。また、アポトーシス誘導をカスパーゼ活性の定量、DNAラダーの検出により解析し、それぞれの誘導レベルとウイルスRNA量との相関を回帰分析により評価した。

【結果と考察】PI牛ではPKR、OAS-1、Mx1とTNFalphaの転写、及びアポトーシスの誘導が有意に認められた。また、回帰分析によりウイルスRNA量とOAS-1、Mx1の転写量やアポトーシス誘導レベルとの間に有意な正の相関が見られた。更に、下痢や発育不良などの症状が認められなかったPI牛は先天性免疫反応の誘導がみられず、ウイルスRNA量も低レベルであったことから、ウイルスRNA量の蓄積に伴う先天性免疫誘導がPI牛における病態に関与している可能性が示唆された。

DV-51 日本産蚊のウエストナイルウイルス増殖・媒介能

○金平克史[1]、白藤浩明[1]、神尾次彦[1]（[1]動物衛生研究所・人獣感染症研究チーム）

ウエストナイルウイルス(WNV)は野鳥から蚊によって人や馬に伝播しウエストナイル熱や脳炎を引き起こす。WNV感染症はヨーロッパ諸国、極東地域をふくむロシアでは散発的に発生があり、近年、北米への感染域拡大がおこった。近い将来、日本にも侵入する可能性があるため、日本産蚊の室内継代株を用いて本ウイルスの感受性を検討した。アカイエカ、ヒトスジシマカ2系統、オオクロヤブカの蚊にWNV NY99株を10^7 PFU/蚊となるように腹部に注入した（注入群）。また、アカイエカ、ヒトスジシマカに感染ジュウシマツを吸血させた（吸血群）。これらの蚊を湿度70−80%、室温25℃で維持し、7日目および14日目に各群の蚊10匹を用いてウイルス力価を測定した。加えて14日目に各群10匹の蚊あたり1匹のマウスを吸血させ媒介試験を行った。媒介試験は各群3回行った。

注入群、吸血群ともに全種類の蚊においてウイルスの増殖が観察された。全ての蚊が7日目で10^6 PFU/蚊 以上、14日目で10^5 PFU/蚊 以上のウイルス力価を示した。媒介試験では、アカイエカ注入、吸血両群、ヒトスジシマカ2系統の注入群、1系統の吸血群では供試したすべてのマウスが8日以内に立毛や後躯麻痺の症状を示し、12日以内に死亡した。死亡したマウスからはWNVが検出された。ヒトスジシマカ1系統の吸血群とオオクロヤブカ注入群では2匹のマウスが同様の経過を示した。感染ジュウシマツを吸血したアカイエカとヒトスジシマカがマウスにウイルスを媒介したことから、野外においてこれら日本産蚊が鳥類から哺乳類への媒介を担う可能性が示された。

DV-52 中空ウイルス粒子を用いたウエストナイルウイルスと日本脳炎ウイルスに対する感染血清の鑑別

○前田潤子[1]、高木弘隆[2]、倉根一郎[3]、高島郁夫[4]、前田秋彦[1]（[1]北大・獣医 プリオン病学講座、[2]感染研・バイオセーフティー管理室、[3]感染研・ウイルス第一部、[4]北大・獣医 公衆衛生）

【背景・目的】日本脳炎ウイルス（JEV）とウエストナイルウイルス（WNV）は同じ血清型群に属するため、両ウイルスの感染を血清学的に区別することは困難である。しかし、WNVの国内侵入をモニタリングするためには、両ウイルスに対する感染歴を把握する必要がある。そこで、本研究ではJEVと WNVの感染により産生される抗体の鑑別法を確立することを目的とした。【材料と方法】WNVの NY株と Eg101株、JEVのJaGAr01株とBeijin株をVero E6細胞で増殖させ、培養上清からウイルス粒子を精製した。各ウイルスの中空ウイルス粒子(SvPs)を発現するベクターを 293T細胞に導入し、培養上清中に放出されるSvPsを精製した。ウイルス粒子、SvPs、感染Vero E6 細胞および遺伝子導入293T細胞を抗原として用いた。また、WNVと JEVの不活化ウイルスをマウスに2回免疫し、最後にウイルスを感染して得られたhyper immune serum (HIS) を抗体として用い、両ウイルスの鑑別の可能性を比較検討した。【結果と考察】ウイルス粒子とSvPs間で、WNVとJEVに対する HISへの反応性に差異は認められず、SvPsをウイルス粒子の代替として使用できると考えられた。ELISA法や IFA法、ウエスタンブロット(WB)法ではWNVとJEVに対するHIS間で交差反応は認められたが、両者を区別することは可能であった。特にWB法では、他の方法に比べ、より特異的に両ウイルスに対するHISを区別することが出来た。以上のことから、JEV あるいはWNVの感染により産生される抗体についても、検査鑑別が可能であると期待できる。

医薬品 研究報告 調査報告

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 20	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	Ramful D, Carbonnier M, Pasquet M, Bouhmani B, Ghazouani J, Noormahomed T, Beullier G, Attali T, Samperiz S, Fourmaintraux A, Alessandri JL. Pediatr Infect Dis J. 2007 Sep;26(9):811-5.	公表国 フランス
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

○チクングニヤウイルス感染症の母子伝播

背景:2005年～2006年に仏領レユニオン諸島でチクングニヤ熱(蚊媒介性アルファウイルス感染)のアウトブレイクが起こった。この流行期間中、母子伝播の可能性が高い早期新生児症例が認められた。

方法:5つの新生児医療部門において後方視的記述的研究を実施した。チクングニヤウイルス感染は、RT-PCR法又は母親と新生児の特異的血清検査にて確認した。出産時に徴候があった場合又は新生児が出生初日に発病した場合に母親のスクリーニングを行った。

結果:新生児38名を登録した。無症候の母親2名を除き、全母親に周産期[範囲:分娩前4日～分娩後1日]の症状が存在した。新生児にはいずれも症状があり、症状は出生後3日～7日(平均4日)に発現した。母体の疾患発症から新生児の疾患発症までの平均期間は、5日間(範囲3～9日間)であった。新生児にもっとも多く見られた臨床徴候は、発熱(79%)、疼痛(100%)、発疹(82%)、末梢浮腫(58%)であった。血小板減少症(76%)、リンパ球減少症(47%)、プロトロンビン低下(65%)、AST上昇(77%)が検知された。合併症には、発作(6)、出血性症候群(6)、血行動態障害(10)が認められた。脳髄液のRT-PCR法は24名中22名で陽性であり、25名中14名に脳MRIの異常所見(白質の病変または実質内出血、あるいはその両方)が認められた。心エコー検査では、心筋肥大(5)、心室機能不全(2)、心膜炎(2)、冠動脈拡張(6)が示された。新生児1名は壊死性腸炎で死亡した。

結論:レユニオン諸島で発生したチクングニヤ熱流行により、周産期母子伝播の罹患率が高い可能性が初めて明らかとなった。

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
2005年～2006年に仏領レユニオン諸島で発生したチクングニヤ熱の大規模流行期間中、母子伝播の可能性が高い早期新生児症例が認められたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。国内でチクングニヤ熱が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

(19)

Mother-to-Child Transmission of Chikungunya Virus Infection

Duksha Ramful, MD, Magali Carbonnier, MD,† Marlène Pasquet, MD,* Brahim Bouhmani, MD,† Jamal Ghazouani, MD,‡ Tahir Noormahomed, MD,§ Gilles Beullier, MD,‖ Tania Attali, MD,* Sylvain Samperiz, MD,* Alain Fourmaintraux, MD,† and Jean-Luc Alessandri, MD**

Background: In 2005–2006 Reunion Island experienced a massive outbreak of chikungunya, a mosquito-borne alphavirus infection. During this epidemic, early neonatal cases were observed with a highly probable mother-to-child transmission.

Methods: A retrospective descriptive study was conducted in 5 neonatal medicine departments. Chikungunya virus infection was confirmed by reverse transcription-polymerase chain reaction or specific serology in mothers and their newborns. Mothers were screened if they presented signs at delivery or if their neonates became ill on the first days of life.

Results: Thirty-eight neonates were enrolled. All mothers, except 2 asymptomatic mothers, presented signs during the perinatal period (range, day(D) −4 to D+1). All neonates were symptomatic and presented symptoms on D3 to D7 (mean, D4). The mean interval between onset of maternal illness and onset of neonatal illness was 5 days (range, 3–9). The most frequent clinical signs in neonates were fever (79%), pain (100%), rash (82%), and peripheral edema (58%). Thrombocytopenia (76%), lymphopenia (47%), decreased prothrombin value (65%), and elevation of aspartate aminotransferase (77%) were detected. Complications included seizures (6), hemorrhagic syndrome (6), and hemodynamic disorders (10). Reverse transcription-polymerase chain reaction in cerebrospinal fluid was positive in 22 of 24 cases, and abnormal findings on brain magnetic resonance imaging (14 of 25) with white matter lesions or intraparenchymal hemorrhages or both were found. Echocardiography (16) showed myocardial hypertrophy (5), ventricular dysfunction (2), pericarditis (2), and coronary artery dilatation (6). One neonate died of necrotizing enterocolitis.

Conclusions: The chikungunya epidemic that occurred on La Reunion Island revealed for the first time the possibility of mother-to-child transmission in the perinatal period with a high rate of morbidity.

Key Words: chikungunya, alphavirus, neonate, pregnancy, mother-to-child transmission
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The islands of the southwest Indian ocean, including La Reunion Island (east of Madagascar), have been exposed since 2005 to an epidemic of chikungunya, an arbovirus infection transmitted by a mosquito of the *Aedes* genus (*Aedes albopictus* in La Reunion Island).[1] The chikungunya virus (CHIKV) is an alphavirus of the Togaviridae family,[2] isolated for the first time in Tanzania and Uganda in 1953.[3,4] It has developed according to an endemic or epidemic mode in sub-Saharan Africa, Southern India, Southeast Asia, and in the Pacific.[5–8] CHIKV is a single-chain linear RNA virus with a diameter of 60–70 nm, enclosed by an envelope.[2]

Clinical signs and symptoms of chikungunya in adults and children are preceded by an incubation period of 4–7 days. CHIKV infection usually presents with sudden onset of high fever and disabling joint and muscle pain (chikungunya is a Swahili word meaning "that which bends up," referring to the stooped walk of patients), often associated with a maculopapular rash and gastrointestinal complaints.[9,10] A hemorrhagic syndrome, generally minor, has also been described, consisting of petechial purpura, epistaxis, or bleeding gums.[11] The natural history of the disease is usually favorable with complete resolution within 1 week. Chronic forms are possible with persistence or recurrence of arthralgia for several months. Complications reported in adults and children are neurologic (meningoencephalitis, polyneuropathy), hemorrhagic,[12–17] and cardiac (pericarditis, myocarditis, cardiac arrhythmias).[18–21] No case of mother-to-child transmission was described before the La Reunion Island epidemic. The youngest child infected by CHIKV reported in the literature before this epidemic was a 21-day-old infant.[15]

The laboratory diagnosis of CHIKV infection is based on 2 types of tests: detection of viral RNA by reverse transcription-polymerase chain reaction (RT-PCR) and IgM- and IgG-specific serology. RT-PCR is positive during the viremic phase lasting for 1 week after onset of symptoms. Specific IgM antibodies are detected by the fifth day after onset of the disease and persist for several weeks to 3 months, whereas specific IgG appear on the 15th day and persist for years.[1]

The first case of chikungunya in La Reunion Island was reported in March 2005. The epidemic was surprising in terms of its magnitude and the development of clinical forms

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From the *Service de Réanimation Néonatale et Pédiatrique, CHD Félix Guyon, Saint-Denis de La Réunion; †Service de Réanimation Néonatale et Pédiatrique, Groupe Hospitalier Sud Réunion; ‡Service de Pédiatrie, Centre Hospitalier Intercommunal Saint-Benoit; §Service de Néonatologie, Clinique Sainte-Clotilde; and ‖Service de Pédiatrie, Centre Hospitalier Gabriel Martin, La Réunion, France.

Address for correspondence: Duksha Ramful, MD, Service de Réanimation Néonatale et Pédiatrique, Centre Hospitalier Départemental Félix Guyon, 97405 Saint-Denis Cedex, La Réunion, France. E-mail: duk@gawab.com.

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that had rarely or never been previously described. The epidemic peak was reached between January 2006 and February 2006 with 46,000 cases per week during this period.[1] At the end of April 2006, the estimated cumulative number of cases was 256,000, ie, 33% of the La Reunion Island population. We report a series of 38 neonates with CHIKV infection during the first week of life after perinatal mother-to-child transmission of the virus. Some cases have already been reported in a previous publication.[22]

PATIENTS AND METHODS

This is a retrospective descriptive study conducted by 5 neonatal medicine departments in public or private hospitals complying with European standards with air-conditioned mosquito-free maternity units. Thirty-eight neonates born between March 2005 and April 2006 were included in this study. Charts were reviewed by 2 investigators.

Definition. The diagnosis of neonatal CHIKV infection was based on RT-PCR detection of the viral genome in the neonate's serum or cerebrospinal fluid (CSF) or both during the first week of life and/or detection of serum anti-CHIKV IgM during the first 15 days of life. The diagnosis of maternal CHIKV infection was based on RT-PCR detection of the viral genome in serum or the presence of serum anti-CHIKV IgM or both. Mothers were screened if they presented signs of chikungunya at delivery or if their neonates became ill on the first days of life. Mother-to-child transmission of CHIKV infection was diagnosed when CHIKV infection was diagnosed in the mother during the perinatal period and when CHIKV infection was diagnosed in her newborn infant during the days after birth, according to the criteria defined above.

Laboratory Methods. Detection of serum anti-CHIKV IgM was performed by an immunocapture method (Mac-ELISA) according to the techniques and reagents of the Centre National de Référence des Arboviruses (National Reference Centre) in Lyon and the Centre National de Référence of the Institut de Médecine Tropicale du Service de Santé des Armées (Armed Forces Institute of Tropical Medicine) in Marseille: capture of IgM by a serum antihuman IgM (I-2386; Sigma, St. Louis, MO), successive addition of CHIKV antigen prepared in cell culture, followed by hyperimmune murine ascites, and revelation of the antibody by peroxidase-marked antimouse conjugate (Sigma, A-0168).

RT-PCR detection of the viral genome was performed after extraction in 200 μL of sample either manually using Viral QIAAmp Minikit (Qiagen, Courtaboeuf, France) or on the MagNa Pur automat (Roche Diagnostic) using the High Pure Viral RNA kit (Roche Diagnostic). RT-PCR was performed with the SuperScript One-Step RT-PCR with Platinium *Taq* kit according to the method of Pastorino et al.[23]

Maternal Data and Outcome of Pregnancies. The following data were recorded: date of onset of symptoms of CHIKV in relation to delivery, fetal heart rate abnormalities, mode of delivery, and birth term.

Neonatal Clinical Data and Complementary Investigations. The following clinical data were analyzed: birth weight, sex, age of onset of signs of illness, type of signs, any complications, and short-term course. Pain was assessed using the EDIN (Echelle Douleur Inconfort Nouveau-Né, neonatal pain and discomfort scale). The following treatments were recorded: analgesia, red blood cell or platelet transfusions, and fresh frozen plasma. Laboratory data recorded, whenever possible, were complete blood count and platelets, serum calcium, hepatic and cardiac enzymes, hemostasis, C-reactive protein, and CSF analysis. Results of the following complementary investigations were analyzed: head ultrasound and brain magnetic resonance imaging (MRI). The MRI protocol comprised T1, T2, and diffusion sequences. In one of the centers, a cardiologic assessment was systematically performed in 16 neonates, comprising laboratory work-up [aspartate aminotransferase, creatine protein kinase, creatine kinase myocardial fraction, and troponin Ic], electrocardiogram, and transthoracic echocardiography.

RESULTS

Diagnosis of CHIKV Infection for Mother-Child Pairs. Thirty-eight neonates presented documented mother-to-child transmission of CHIKV infection. The diagnosis of maternal infection was confirmed by RT-PCR detection of CHIKV (21 of the 26 mothers tested) or by the presence of specific anti-CHIKV IgM (21 of the 24 mothers tested) or both. The diagnosis of neonatal infection was confirmed by RT-PCR (24 of the 24 neonates tested) or by the presence of specific IgM (26 of the 30 neonates tested) or both.

Maternal History. Thirty-six mothers of CHIKV-infected infants presented signs of chikungunya during the perinatal period, and onset of illness ranged from 4 days prepartum to the day after delivery (mean, −0.7 days in relation to delivery). Eighteen mothers (50%) developed clinical signs on the day of delivery. Two mothers (5%) had an asymptomatic form of the disease.

Neonatal Clinical Features. Clinical data are summarized in Table 1. Twenty-six (68%) neonates were delivered vaginally and cesarean section was performed in 12 cases (32%). Fetal heart rate abnormality was observed in 17 cases (45%). There

TABLE 1. Clinical and Laboratory Data for the 38 Neonates Studied

Observation	N (%)
Clinical	
EDIN score >3	38 (100)
Fever	30 (79)
Rash	31 (82)
Edema	22 (58)
Diarrhea	12 (32)
Seizures	6 (16)
Hemorrhagic syndrome	6 (16)
Hemodynamic disorders	10 (26)
Death	1 (3)
Laboratory	
Thrombocytopenia (<150 × 10^9/L)	29 (76)
Thrombocytopenia (<50 × 10^9/L)	12 (32)
Lymphopenia (<1 × 10^9/L)	18 (47)
Hypocalcemia (<1.9 mmol/L), n = 27	5 (19)
Prothrombin level <50%, n = 26	17 (65)
ASAT >50 IU/L, n = 30	23 (77)

EDIN indicates Echelle Douleur Inconfort Nouveau-Né, neonatal pain and discomfort scale.

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were 22 boys and 16 girls. Mean gestational age was 38 weeks (range, 35–41) and mean birth weight was 3000 g (range, 1900–4010). In all infants, the first symptoms appeared between the third day of life (D3) and D7 (mean, D4). The mean interval between onset of maternal clinical signs and onset of neonatal clinical signs was 5 days (range, 3–9). Mean duration of fever was 2 days (n = 30, range, 1–8) and pain lasted for an average of 8 days (range, 2–22). Thirty-one infants developed a rash with a fairly characteristic course: initial erythroderma followed by desquamation of the extremities and, subsequently, a brownish dyschromia of the limbs and sometimes the face. Severe peripheral edema was observed in 22 cases. Most infants (n = 33) presented feeding problems requiring temporary tube feeding and 12 had diarrhea. Hemorrhagic manifestations observed were 3 gastrointestinal hemorrhages (with 1 case of necrotizing enterocolitis), 2 important intracerebral hemorrhages, and 1 case of hemorrhagic conjunctivitis. Neurologic features consisted of hypotonia (n = 17), coma, brief seizures, or status epilepticus (n = 6) during the first days of the disease. Ten neonates presented initial hemodynamic disorders.

Laboratory Tests and Complementary Investigations in Neonates. Laboratory data are summarized in Table 1. The laboratory abnormalities most frequently observed were thrombocytopenia, lymphopenia, decreased prothrombin concentration, and elevation of aspartate aminotransferase. CSF analysis was performed in 24 infants and biochemical and cytologic analysis were normal in 12 nonhemorrhagic cases. RT-PCR detection of CHIKV in CSF was positive in 22 of the 24 cases tested, including in nonhemorrhagic CSF. Head ultrasound performed in 26 neonates was pathologic in 13 cases. Precise data were available for only 7 neonates. In 5 neonates, head ultrasound demonstrated lenticulothalamostriatal vasculitis either isolated or associated with frontal or parietal periventricular hyperechogenicity (n = 4), or cerebral edema with reduction of the vascular resistance index (n = 1). MRI was performed in 25 neonates during the acute phase of the disease with abnormal findings in 14 infants. Scattered white matter lesions were found in 11 cases, including the corpus callosum in 5 cases. These lesions presented a high-intensity signal on T2-weighted sequences with reduction of the apparent diffusion coefficient (ADC) on diffusion sequences. MRI was repeated after 1 month in 8 of these 11 cases, demonstrating signal inversion on the diffusion sequence with increased ADC. Areas of intraparenchymal hemorrhage within these scattered white matter lesions were observed in 6 cases and bilateral petechial lesions were found in 4 cases. No gray matter lesions were observed. All neonates presenting convulsions had abnormal imaging.

In the group of infants undergoing systematic cardiovascular investigations (n = 16), 6 had abnormalities (Table 2). Coronary artery dilatation corresponded to unilateral or bilateral hyperechoic coronary arteries with thickened walls without aneurysmal features.

Treatment. Treatment of CHIKV-infected neonates was symptomatic. All infected neonates were admitted to a neonatal care unit or neonatal intensive care unit. The mean hospital stay was 16 days (range, 3–47). Level I analgesics (paracetamol) were prescribed in 12 cases, whereas the other 26 infants required level 2 (nalbuphine) or level 3 (morphine) analgesics. Mechanical ventilation was necessary in 9 infants (24%) with serious neurologic manifestations (status epilepticus) or multiorgan failure. Platelet (n = 9) and/or fresh frozen plasma (n = 7) transfusions were performed in neonates with hemorrhagic complications. Neonates with hemodynamic disorders required volume expansion (n = 5) and/or vasopressor amines (n = 4). Two neonates received red blood cell transfusion. One neonate died on D6 because of necrotizing enterocolitis with gastrointestinal hemorrhage and *Klebsiella pneumoniae* septicemia.

DISCUSSION

Perinatal Mother-to-Child Transmission of the CHIKV. Our series demonstrates cases of mother-to-child transmission of CHIKV infection that has never been described during previous epidemics. Mother-to-child transmission of CHIKV is highly probable for the following reasons: (1) all infected neonates were born to mothers with documented chikungunya during the perinatal period; (2) the interval between the onset of maternal and neonatal symptoms was compatible with the CHIKV incubation period; (3) no cases of such early neonatal infection were observed during the La Reunion Island epidemic without concomitant maternal infection during the days preceding delivery. The earliest cases of chikungunya observed in neonates not born to an infected mother

TABLE 2. Clinical and Complementary Cardiologic Data for 6 Cases

Case	Clinical Signs	ECG	Echocardiography	AST	Laboratory		
					CK	CKMB	TIc
1	Severe pain	RD	LVH, LVD, right/left coronary artery dilatation	250	1385	148	0.13
2	Bradycardia/desaturation, severe pain	RD	Pericarditis, LVH, right coronary artery dilatation	224	460	49	0.1
3	Heart failure, collapse	RD	Pericarditis, LVH, LVD, PHT, right/left coronary artery dilatation	172	236	62	0.15
4	Severe pain, tachycardia	RD, SVT	Right coronary artery dilatation	123	306	66	0.1
5	Severe pain, malaise	RD	LVH, right/left coronary artery dilatation	136	1417	74	0.11
6	Heart failure, collapse	RD	LVH with septal dyskinesia, right/left coronary artery dilatation	161	1284	125	1.64

RD indicates repolarization disorders; SVT, supraventricular tachycardia; LVH, left ventricular hypertrophy; LVD, left ventricular dysfunction; PHT, pulmonary artery hypertension; AST, aspartate aminotransferase (IU/L); CK, creatine kinase (IU/L); CKMB, creatine kinase, myocardial fraction (IU/L); TIc, troponin Ic fraction (ng/mL); ECG, electrocardiography.

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occurred after D9 of life and probably corresponded to infections transmitted by mosquito bites soon after discharge from the maternity unit. The hypothesis of neonatal infection acquired after birth by mosquito bite during the first few hours of life needs to be considered, but, in our opinion, is extremely unlikely in European-standard air-conditioned mosquito-free maternity units.

Mother-to-child transmission of other alphaviruses has been reproduced experimentally in animals for Venezuelan equine encephalitis virus and the Semliki Forest virus[24,25] and has been described in humans for Western equine encephalitis and Venezuelan equine encephalitis viruses and is suspected for the Ross River virus.[26,27] Neonatal dengue virus infections (Flaviviridae family) with hemorrhagic complications and death have been described after perinatal transmission, with a chronology resembling that of our cases.[28]

The exact mechanisms of mother-to-child transmission of CHIKV have yet to be elucidated. Currently available data support the hypothesis of mainly perinatal transmission of CHIKV, which seems to be high, close to 50% (personal unpublished data). In the case of maternal infection during pregnancy, but occurring well before delivery, the risk of vertical transmission seems to be extremely low as only 3 cases of fetal deaths at the beginning of pregnancy have been reported during the La Reunion epidemic, and asymptomatic newborns do not seem to be infected.[29,30] Contamination during passage through the genital tract is possible, but no protective effect of cesarean section is noted in our series. Transplacental transmission shortly before delivery is the more likely mode of transmission via mother-to-child microtransfusions when the mother presents positive viremia.

Uncomplicated Neonatal Forms. Twenty-three neonates (61%) presented clinical and laboratory features of chikungunya similar to the classic form observed in adults and the disease resolved spontaneously in several days to 2 weeks. However, the benign nature of classic neonatal forms is only relative, as all CHIKV-infected neonates had to be admitted to a neonatal care unit, because of the need for fairly intensive symptomatic treatment. Furthermore, intrathecal presence of the CHIKV genome and disturbances of the cardiovascular and neurologic work-up were observed in some infants with no obvious severe complications.

Complicated Neonatal Forms. Fifteen neonates (39%) presented complicated or serious forms of chikungunya with hemorrhagic, cardiac, and neurologic manifestations.

The most serious hemorrhagic syndromes were observed in neonates with severe thrombocytopenia, disseminated intravascular coagulation, and impaired liver function. CHIKV is not usually considered to be a classic agent of "hemorrhagic fever," but such an effect has been suggested during epidemics in Southeast Asia and India.[11]

Varying degrees of severity of neurologic complications were described during the Indian epidemic of 1964: disorders of consciousness, seizures, meningeal syndromes, meningoencephalitis, transient ophthalmoplegia, and polyneuropathy.[13–15] The mechanisms of neurologic lesions have not been fully elucidated. White matter signal inversion on diffusion sequences and ADC mapping on MRI reflects the transformation of early cytotoxic edema into vasogenic edema during an interval of 1 month. No direct culture of CHIKV in CSF and no anatomopathologic examination were performed, and so the diagnosis of CHIKV viral encephalitis was not formally confirmed. Long-term follow-up of these infants is necessary to assess their neuromotor and sensory development.

Acute cardiac lesions have also been described in the course of CHIKV infection.[18–21] However, these reports were published before the age of modern imaging, and the respective roles of dengue virus and CHIKV were not always clearly distinguished. The rare pediatric series of CHIKV infection have not reported any cardiovascular complications, apart from 1 case of collapse followed by death in a 3-year-old child.[15] Coronary dilatation during other viral infections such as cytomegalovirus has recently been reported[31] but the precise mechanisms of coronary dilatation during CHIKV infection, especially the possibility of vasculitis, have not been elucidated.

Chikungunya is an arbovirus infection, which, before the La Reunion Island epidemic, was considered a benign disease. Several reasons can be proposed to explain the development of complicated forms of the disease, bearing in mind that a number of serious forms have been previously described but subsequently forgotten. The epidemic occurred on a limited territory equipped with an advanced health care and epidemiologic surveillance system allowed exhaustive identification of serious forms. This was a large-scale epidemic affecting a nonimmune population. Finally, the hypothesis of a genomic evolution of CHIKV resulting in increased virulence has also been proposed.[32]

CONCLUSIONS

The chikungunya epidemic that occurred on the La Reunion Island in 2005–2006 revealed for the first time the possibility of perinatal mother-to-child transmission of CHIKV with a high rate of neonatal morbidity. Knowledge about the clinical course along with rapid confirmation with virologic tests lead to appropriate management that can limit complications.

The absence of specific treatment justifies individual protective measures in pregnant women, particularly close to term: approved insect repellents, mosquito nets. The experience acquired in La Reunion Island and ongoing studies may be useful in other latitudes, especially as the *A. albopictus* mosquito, vector of chikungunya, is present in many regions of Europe and North America. The example of recent West Nile virus epidemics in North America demonstrates that arbovirus infections can affect new territories in nontropical zones.[33]

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研究報告の概要

○イタリア、ラヴェンナ県でのチクングニヤ熱発生

チクングニヤ熱は、アフリカ、東南アジア、インド亜大陸で土着感染しており、2005年以来、大規模な流行がインド洋諸島とインドから報告されているが、これまでヨーロッパ地域内での蚊による感染伝播は発生していなかった。2007年8月中に、イタリアのエミリア・ロマーニャ州ラヴェンナ県衛生当局は、ラヴェンナ近郊の村で異常に多数の発熱患者発生を検知した。臨床・疫学調査から、アルボウイルスが原因であると示唆され、血清学的検査およびPCR法でチクングニヤ熱と確定された。加えてヒトスジシマカからもPCR法によりチクングニヤウイルスが確認された。診断基準は、高熱および関節痛に皮疹もしくは脱力を伴う患者とし、流行地域との関連が不明な症例は、検査によって診断を確定した。

患者数:2007年9月4日までに合計197名の患者が報告され、うち166名が症例定義を満たした。147名は初発感染地区から、19名は近隣の二次感染地区から報告された。残りの31名は、更なる疫学調査や検査による診断確定が必要とされた。初発患者は、インド亜大陸の感染地域から6月21日にイタリアに入国した外国人男性と考えられた。感染のピークは8月の第3週だった。他の散発例が現在近隣地区で確認されているが、初発感染地区では感染は沈静化に向かっている。

臨床所見:大部分の患者の症状は軽症で自己限定的である。ほとんどの患者で発熱は2、3日で治まり、50%以上で斑状発疹が見られた。関節痛は強く発熱が治まった後も持続することがあった。死亡は1名のみで、重篤な基礎疾患がある83歳男性だった。

感染対策:8月29日に、開業医と病院の救急部を拠点とした能動的サーベイランスシステムを立ち上げた。対策として、全患者の住居周囲100m以内での殺虫剤使用、ベクター駆除への積極的な参加の呼びかけ及び感染症情報提供が実施された。感染地区では血液、臓器および組織の提供が一時停止されている。

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
イタリア、ラヴェンナ県で、国内に生息する蚊によってチクングニヤ熱の感染伝播が発生したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。国内でチクングニヤ感染が確認されたため、渡航歴確認の徹底を図っている。また、チクングニヤ熱の既往歴がある場合、治癒後6ヵ月間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

An outbreak of chikungunya fever in the province of Ravenna, Italy

R Angelini[1] AC Finarelli[2], P Angelini[2], C Po[2], K Petropulacos[3], P Macini[2], C Fiorentini[4], C Fortuna[4], G Venturi[4], R Romi[4], G Majori[4], L Nicoletti[4], G Rezza[4], A Cassone (cassone@iss.it)[4]

1. Dipartimento Sanità Pubblica, Azienda Unità Sanitaria Locale (Department of Public Health, Local Health Unit), Ravenna, Italy
2. Servizio di Sanità Pubblica, Regione Emilia-Romagna, Bologna, Italy
3. Servizio Presidi ospedalieri, Regione Emilia-Romagna, Bologna, Italy
4. Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

Chikungunya fever is a viral disease transmitted by *Aedes spp* mosquitoes. The infection is endemic in parts of Africa, South-east Asia and on the Indian sub-continent. Since 2005, large outbreaks have been reported in several islands in the Indian Ocean and in India [1]. Travellers from areas affected by chikungunya have been diagnosed with chikungunya fever in several European countries, including Italy [2], but local transmission involving mosquitoes has not occurred so far.

During the month of August, local health authorities of the province of Ravenna, Region Emilia-Romagna, Italy, detected an unusually high number of cases of febrile illness in Castiglione di Cervia and Castiglione di Ravenna, two small villages divided by a river. At the end of the month, clinical and epidemiological investigations carried out by the local Health Units in collaboration with the Region and the Reference Laboratory of the Istituto Superiore di Sanità in Rome, suggested an arbovirus as the possible cause of the outbreak. Serological testing and PCR confirmed the diagnosis of chikungunya fever. In addition, the chikungunya virus was detected by PCR in *Aedes albopictus*, which is considered to be the most likely vector for this outbreak.

The case definition used includes high fever and joint pain and/or rash and/or asthenia, and, for cases with no apparent link with the two initially affected villages, or with areas affected by secondary clusters, laboratory confirmation.

Number of cases

To date (4 September 2007), a total of 197 cases have been reported. Of these, 166 fulfil the case-definition criteria: 147 are from the initial outbreak area of Castiglione di Cervia or Castiglione di Ravenna, whereas 19 are from secondary clusters in neighbouring suburbs of the towns of Cesena (13 cases) and Cervia (six cases). The remaining 31 suspected cases need further epidemiological investigation (i.e. affected areas visited in the last 14 days) and/or laboratory confirmation. These cases, with no apparent link with the main affected areas, are scattered throughout the Region; their blood samples have not been tested yet.

The index case is believed to be a foreigner coming from an affected area in the Indian subcontinent and not resident in Castiglione. He arrived in Italy on June 21 and developed symptoms two days later when he was in Castiglione di Cervia. The peak of the epidemic curve occurred during the third week of August. Other sporadic cases have been recently detected in neighbouring areas, but the epidemic curve shows a decreasing trend in Castiglione di Cervia and Castiglione di Ravenna.

Clinical presentation

In the large majority of the patients, the disease was mild and self-limiting. Preliminary data from Castiglione show that fever lasted for a few days in most patients and a macular rash appeared in more than 50% of cases; however, arthralgia was intense and often persistent even after the abatement of fever. Only one death occurred, in an 83-year-old man with severe underlying conditions.

Control measures

An active surveillance system based on general practitioners and hospital emergency units was set up in the whole Region on 29 August. Implemented control measures include the use of insecticides (pyrethroids and antilarval products) in public as well as private sites within 100 metres around the residence of all confirmed and suspected cases, and communication to the public to inspire active involvement in vector control measures and general health education. A protocol on how to measure the efficacy of the control measures is being implemented.

A surveillance system for monitoring *Aedes albopictus* distribution has been active in the whole of the Emilia-Romagna Region since 2006. A surveillance for chikungunya infection in *A. albopictus* is being considered. Blood, organ and tissue donation has been suspended in the affected area, i.e. Ravenna municipality, Cervia, Cesena, Cesenatico.

References:

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2. Beltrame A, Angheben A, Bisoffi Z, Monteiro G, Marocco S, Calleri G *et al.* Imported chikungunya infection, Italy [letter]. Emerg Infect Dis [serial on the Internet]. 2007 Aug. Available from: http://www.cdc.gov/EID/content/13/8/1264.htm

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 7. 24	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ABC Newsletter. 2007 Jul 20.	公表国	
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			オーストラリア	

研究報告の概要

○感染症最新情報:デング
オーストラリアのクイーンズランド州北部におけるデング熱アウトブレイクにより、オーストラリア赤十字では、流行地に滞在した人から供血された血液製剤の廃棄を余儀なくされた。局地的アウトブレイクは3月末にTownsville南部で始まったが、5月14日まで新規症例は記録されなかった。赤十字のスポークスマンは、供血以前にTownsville南部を訪れた供血者由来の赤血球・血小板製剤を破棄するが、血漿製剤は使用できると伝えた。また、クイーンズランドの予防戦略と封じ込め戦略により、デング熱アウトブレイクが血液事業に及ぼす影響は、アウトブレイクが起こるたびに小さくなってきていると語った。供血制限は、アウトブレイクの終息が正式に宣言されるまで継続される。Townsville Tropical Population Health Centreは(新規症例が報告されなくなって3ヶ月となる)8月6日までかかるだろうとの見解を示した。

使用上の注意記載状況・その他参考事項等

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

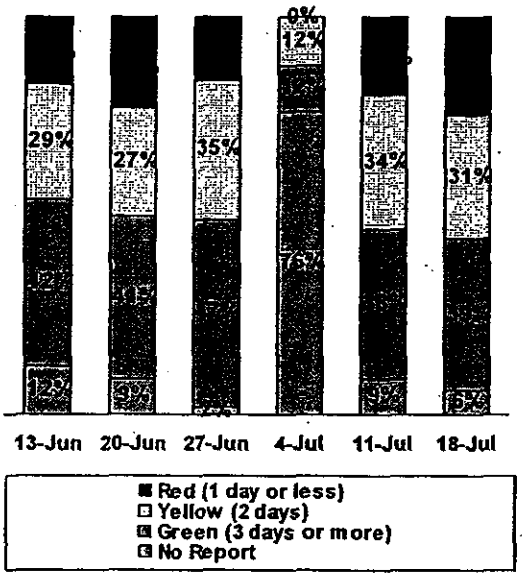
血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
オーストラリアのクイーンズランド州北部におけるデング熱アウトブレイクにより、オーストラリア赤十字は、流行地に滞在した人から供血された血液製剤の廃棄を余儀なくされたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。問診でデング熱の既往があった場合には、治癒後1ヶ月間献血不可としている。今後も引き続き情報の収集に努める。

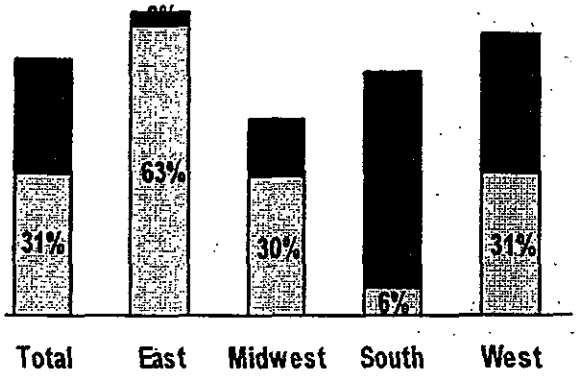
(12)

STOPLIGHT: Status of America's Blood Centers' Blood Supply

Total ABC Red Cell Inventory



Percent of Regional Inventory at 2 Days Supply or Less, July 18, 2007



Daily Updates are available at:
www.americasblood.org

INFECTIOUS DISEASE UPDATES:

DENGUE

A dengue fever outbreak in north Queensland, Australia has forced the Red Cross Blood Service to discard some blood components donated by people who spent time in areas affected by the disease. The local outbreak began in South Townsville at the end of March, but no new cases have been recorded since May 14. A spokesman for the Red Cross Blood Service, Rudy Bell, said the service has discarded red cells and platelet components from donors who have visited South Townsville before giving blood. However, he said the plasma component is still able to be used. "Thanks to both the prevention and containment strategies of the Queensland Health dengue fever management plan, the impact of a dengue fever outbreak on the blood service is consistently being reduced each time there is one," he said. Restrictions on blood donations will continue until the outbreak is declared officially over. Townsville Tropical Population Health Centre said that could take until August 6 (no new cases will have been recorded for three months). (Source: ABC Australia, 7/12/07)

West Nile virus (WNV) has claimed the life of an elderly woman in Kern County, California, making her the first to die this year in that state from complications associated with the virus. "This unfortunate death reminds us that we must take precautions to protect ourselves and our families from mosquito bites. Even though the likelihood of serious illness from West Nile virus is low for most people, all Californians should take every precaution to reduce their risk of exposure," Mark Horton, MD,

(continued on page 15)

医薬品　研究報告　調査報告書

識別番号・報告回数		報告日	第一報入手日 2007年8月10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. 2007;56:785-789	公表国 米国 メキシコ	
販売名（企業名）	別紙のとおり				

研究報告の概要

問題点：米国テキサス州南部、メキシコとの国境付近におけるデング熱のアウトブレイク

米国 Texas 州南部、メキシコとの国境付近ではデング熱の散発的、局所的発生が報告されているが、デング出血熱に関しての発生報告はない。今回、2005 年 7 月にデング出血熱症例 1 例が Texas・Brownsville の住民において報告され、2005 年 8 月に、隣接するメキシコ Tamaulipas 州の保健当局は、デング出血熱症例 223 例（17.8%）を含むデング熱症例 1251 例が発生しているデング熱のアウトブレイクを報告した。このアウトブレイクの特徴を明らかにするため、メキシコの保健当局、米 CDC などは臨床的および疫学的調査を実施した。調査により、Texas-Tamaulipas の国境でのデング熱のアウトブレイクに関連したデング出血熱症例の割合の増加が確認された（2000－2004 年はデング熱症例 541 例が報告され，デング出血熱と分類されたのは 20 例(3.7%)であった)。

Tamaulipas 州では、デング出血熱症例は 2000 年 2.2%から 2006 年には 23.4%に増加しており、最近、Texas 州でのデング出血熱発症が報告されるようになった。今後、国境付近でのデング熱に関する調査を強化するとともに、医療関係者はデング出血熱に関する認識を高めるとともに、治療法を習得すべきと考える。

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。

使用上の注意記載状況・その他参考事項等

記載なし

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗 HBs 人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免役グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン 20 “化血研”、②献血アルブミン 25 “化血研”、③人血清アルブミン “化血研” *、④ “化血研” ガンマーグロブリン、⑤献血静注グロブリン “化血研”、⑥献血ベニロンーⅠ、⑦ベニロン、⑧注射用アナクトC2,500 単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン “化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン 20%化血研*、⑱アルブミン 5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP 1500 注射用
報告企業の意見	デング熱は、ネッタイシマカなどの蚊が媒介するデングウイルスの感染症であり、基本的には自然回復傾向の強い軽症感染症であるが、血小板減少による重症型の出血傾向を伴ったデング出血熱（DHF）に移行することがある。DHF は出血、血小板減少、血管透過性増加により致死的ショックを来す重症疾患である。デングウイルスは、フラビウイルス科に属する直径 40-50nm の球形ウイルス（1本鎖 RNA）でエンベロープを有している。日本国内にはデングウイルスは常在せず、デング熱患者は全て輸入感染症である。本研究報告は、ヒトの血液を介してデングウイルスが感染したとの報告ではなく、デングウイルスが血液を介してヒトに感染したという報告も見出し得ていないが、デングウイルスは感染後ウイルス血症を起こすことから、血液を介してウイルス感染する可能性を完全に否定できないため本報告を行った。 仮にウイルスが原料血漿に混入していたとしても、弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。 各製造工程のウイルス除去・不活化効果は、「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）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A 型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したデングウイルスは、エンベロープの有無、核酸の種類等からモデルウイルスとしては BVDV が該当すると考えられるが、上記バリデーションの結果から、BVDV の除去・不活化効果を有することを確認している。 また、これまでに当該製剤によるデングウイルス感染の報告例は無い。 以上の点から、当該製剤はデングウイルス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

MMWR
Weekly
August 10, 2007 / 56(31);785-789

Dengue Hemorrhagic Fever --- U.S.-Mexico Border, 2005

Please note: An erratum has been published for this article. To view the erratum, please click here.

Dengue fever is a mosquito-transmitted disease caused by any of four closely related virus serotypes (DEN-1, DEN-2, DEN-3, and DEN-4) of the genus *Flavivirus*. Infection with one of these serotypes provides lifelong immunity to the infecting serotype only. Therefore, persons can acquire a second dengue infection from a different serotype, and second infections place them at greater risk for dengue hemorrhagic fever (DHF), the more severe form of the disease (*1*). DHF is characterized by bleeding manifestations, thrombocytopenia,* and increased vascular permeability that can lead to life-threatening shock (*2*). In south Texas, near the border with Mexico, sporadic, locally acquired outbreaks of dengue fever have been reported previously; however, on the Texas side of the border, these outbreaks have not included recognized cases of locally acquired DHF in persons native to the area. In July 2005, a case of DHF was reported in a resident of Brownsville, Texas (Figure 1). In August 2005, health authorities in the neighboring state of Tamaulipas, Mexico, reported an ongoing dengue outbreak with 1,251 cases of dengue fever, including 223 cases (17.8%) of DHF. To characterize this dengue outbreak, the Texas Department of State Health Services (TDSHS), Mexican health authorities, and CDC conducted a clinical and epidemiologic investigation. This report summarizes the results of that investigation, which determined that the percentage of DHF cases associated with dengue fever outbreaks at the Texas-Tamaulipas border has increased. Health-care providers along the U.S. border with Mexico should be vigilant for DHF and familiar with its diagnosis and management to reduce the number of severe illnesses and deaths associated with outbreaks of dengue fever.

Autochthonous DHF Case Report

On June 24, 2005, a woman from Brownsville, Texas, had acute onset of fever, chills, headache, nausea, vomiting, abdominal pain, arthralgia, and myalgia. As a youth, the patient had resided across the border in the city of Matamoros in Tamaulipas, Mexico; however, she had been a Brownsville resident for 16 years with the exception of 1 year in Houston, Texas. After she became ill, the woman crossed the border into Matamoros for the first time in approximately 2 months, where she visited a clinician and was given antibiotics. On June 28, the woman was hospitalized in Matamoros with a diagnosis of probable dengue fever and urinary tract infection. During her 3-day hospitalization in Mexico, she had thrombocytopenia (62,000 platelets/mm^3) but no hemorrhagic manifestations; she was treated with fluids and antibiotics and discharged.

On July 1, the woman reentered the United States and sought treatment for continued fever, chills, vomiting, and abdominal pain. She was admitted to a hospital in Brownsville, Texas, where her blood pressure was 94/70 mm Hg, and laboratory testing indicated proteinuria, hematuria, and a further decrease in platelet count (43,000/mm^3). She was given antibiotics for suspected partially treated urinary tract infection and fluids for dehydration. During her hospital stay, the patient's platelet count dropped to 39,000/mm^3 and albumin to 2.9 g/100 mL; a fecal

occult blood test was positive, and pleural effusion was noted on ultrasound. Upon discharge on July 4, her platelet count had increased to 118,000/mm^3. The woman was discharged with a diagnosis of possible murine typhus or viral infection and instructions to take a course of doxycycline.

Although the woman's clinical characteristics (i.e., acute fever, platelet count ≤100,000/mm^3, evidence of bleeding [hematuria and fecal occult blood] and plasma leakage) were consistent with World Health Organization (WHO) criteria for DHF (Box) (*2*), dengue was not diagnosed at the Brownsville hospital. Subsequently, results from a July 3 serum sample from the woman obtained by the regional Texas Border Infectious Disease Surveillance (BIDS) project tested positive for dengue immunoglobulin M (IgM) by enzyme-linked immunosorbent assay (ELISA) and had an elevated titer of immunoglobulin G (IgG) antibodies to dengue fever (1:655,350); this was interpreted as indicative of a secondary dengue infection (*1*).

Outbreak Investigation and Response

Dengue fever case finding. On August 27, 2005, Tamaulipas State Health Services reported to TDSHS that an outbreak of dengue fever in the border state had grown to 1,251 cases that met the Mexico case definition (i.e., fever and at least two of the following symptoms: headache, myalgia, arthralgia, and rash). Using WHO criteria for DHF, Tamaulipas health authorities had classified 223 (17.8%) of the cases as DHF, an increase in the percentage classified as DHF from 2000--2004, when 541 dengue fever cases were reported, including 20 cases (3.7%) classified as DHF.†

In October, investigators in Texas and Tamaulipas began conducting expanded outbreak case finding, including active surveillance in local hospitals, with laboratory testing encouraged for patients with undifferentiated fever as part of the BIDS project. In Cameron County, Texas, where Brownsville is the county seat, TDSHS identified 24 additional cases of laboratory-confirmed dengue fever§, including two additional cases of locally transmitted dengue fever and 22 cases associated with travel to Mexico; the cases had been reported during August--November (Figure 2). The serotype most commonly associated with the outbreak was identified as DEN-2 (i.e., 27 of 28 viral isolates in Tamaulipas). Molecular analysis of isolates at CDC indicated that the circulating strain of DEN-2 was one previously associated with DHF in the Americas region (*4,5*). Plotting reports of cases by week determined that the border outbreak peaked in October and substantially subsided by December (Figure 2).

DHF case finding. In December, investigators reviewed medical records of 129 patients who had been hospitalized and reported to public health authorities with both clinical and laboratory evidence of dengue fever, including 25 persons treated at three Cameron County hospitals and 104 treated at three hospitals in Matamoros. Fifty-nine percent of the patients were female. Ages ranged from 30 to 76 years (median 47.5 years) among the Cameron County cases and from 7 to 70 years (median 36.0 years) among the Matamoros cases. In addition to fever, 82% had myalgia, 78% headache, 41% abdominal pain, 23% rash, and 19% had underlying chronic diseases. No fatalities were recorded. A total of 16 (64.0%) of the 25 dengue cases from Cameron County and 34 (32.7%) of the 104 cases from Matamoros met WHO criteria for DHF (Box). Eleven of the 50 DHF cases, including one from Cameron County, were classified as WHO grade III, or dengue shock syndrome, with early or mild evidence of hypotension or shock. The remaining 39 DHF cases were classified as WHO grade II.¶

Serosurveys. Because many dengue infections are asymptomatic, and most ill persons likely do not seek medical attention, investigators conducted serosurveys to assess the incidence of dengue infection in the populations of Matamoros and Brownsville. Serosurveys also enable estimation of the population susceptible to second dengue infections and DHF. For the serosurveys, a two-stage cluster design was used to obtain a representative sample of households from Brownsville and Matamoros (*6*). Thirty census tracts were selected systematically from each city after stratifying by income. Four households were selected from each census tract after mapping and selecting a

random start point and random direction for sampling.

At each participating household, all residents present and aged ≥5 years were asked to provide a blood sample and demographic information. Serum samples were tested for IgM and IgG antibodies to dengue virus by ELISA. The seroincidence of recent dengue infection was defined by IgM antibodies ≥0.2 optical density (OD). Seroprevalence was defined as the presence of IgG antibodies ≥1:40. Data were weighted to reflect probability of selection, taking into account the population and numbers of households per census tract and size of household.

In Matamoros, 240 households were visited during December 5--10, and 143 (59.6%) had residents at home. Blood samples were obtained from 131 persons in 111 homes. Of these samples, 30 were anti-dengue IgM positive (weighted prevalence: 22.8%; 95% confidence interval [CI] = 13.3%--32.3%), and 101 were IgG positive (weighted prevalence: 76.6%; CI = 64.7%--88.5%). In Brownsville, 346 households were visited during December 12--15, and 161 (46.5%) had residents at home. Blood samples were obtained from 141 persons in 118 homes. Of these samples, four were anti-dengue IgM positive (weighted prevalence: 2.5%; CI = 0%--5.4%) and 47 were IgG positive (weighted prevalence: 38.2%; CI = 26.7%--49.8%). Of 24 Brownsville participants with no history of travel outside the United States, six (25%) were seropositive for IgM or IgG antibodies to dengue.

Reported by: *A Abell, PhD, B Smith, MD, M Fournier, MD, Texas Dept of State Health Svcs, Harlingen, Texas; T Betz, MD, L Gaul, PhD, Texas Dept of State Health Svcs, Austin, Texas; JL Robles-Lopez, MD, CA Carrillo, MD, Jurisdicción Sanitaria No. III de Matamoros, Matamoros, Tamaulipas; A Rodríguez-Trujillo, MD, Servicios de Salud de Tamaulipas, Cd. Victoria, Tamaulipas; C Moya-Rabelly, MD, Mexico Section of the US-Mexico Border Health Commission, Tijuana, Baja California; O Velasquez-Monroy, MD, C Alvarez-Lucas, MD, Centro Nacional de Vigilancia Epidemiológica y Control de Enfermedades, Mexico, DF; P Kuri-Morales, MD, L Anaya-Lopez, MD, Dirección General de Epidemiología, México, DF. M Hayden, PhD, National Center for Atmospheric Research, Boulder, Colorado. E Zielinski-Gutierrez, DrPH, J Muñoz, PhD, M Beatty, MD, I Sosa, Div of Vector-Borne Infectious Diseases, National Center for Zoonotic, Vector-Borne, and Enteric Diseases; S Wenzel, MPH, Career Development Div, Office of Workforce and Career Development; M Escobedo, MD, S Waterman, MD, Div of Global Migration and Quarantine, National Center for Preparedness, Detection, and Control of Infectious Diseases; M Ramos, MD, BK Kapella, MD, H Mohammed, PhD, R Taylor, PhD, J Brunkard, PhD, EIS officers.*

Editorial Note:

DHF incidence has increased in the Western Hemisphere in Latin America and the Caribbean during the past two decades (*3*). Over this period, the epidemiology of dengue in Mexico and Texas has changed. Since 1995, when all four dengue serotypes were identified as circulating in Mexico, an increasing percentage of reported dengue cases in Mexico have been DHF (*7*). In the Mexican border state of Tamaulipas, all four serotypes were first reported in circulation in 1995, and the proportion of reported DHF cases increased from 2.2% in 2000 to 23.4% in 2006. In south Texas, all dengue serotypes have circulated periodically (*3,8*), but locally acquired DHF has been reported only recently (*9*). The first report of locally acquired DHF in Texas, published in 2004, described a fatal case involving a woman originally from Southeast Asia (*9*). She presumably had acquired her first dengue infection in Asia and her second dengue infection in Val Verde, Texas, near the U.S.-Mexico border. However, the DHF case described in this report is the first in a Texas resident who was native to the U.S.-Mexico border area. Case-finding activities during the dengue outbreak identified 15 additional DHF cases on the Texas side of the border.

Entomologic, serologic and virologic conditions are now such that locally acquired DHF can occur in south Texas. The principal dengue vector, the *Aedes aegypti* mosquito, is well established in south Texas, as is *Aedes albopictus*, which also is capable of transmitting dengue

(*7,10*; TDSHS, unpublished data, 2007). The finding that 38% of surveyed Brownsville residents have IgG antibodies to dengue indicates that a substantial proportion of the city population has been infected with the dengue virus and might be more susceptible to DHF if they receive a second infection with a heterologous dengue serotype. The presence in Brownsville of multiple dengue serotypes since 1980 might increase the likelihood for secondary dengue infections from a different serotype and increase the risk for DHF.

The findings in this report are subject to at least two limitations. First, more comprehensive laboratory testing on the U.S. side of the border during the 2005 outbreak likely accounted for the greater percentage of patients meeting DHF criteria among hospitalized dengue patients in Cameron County compared with Matamoros. As such, the results for these two sites are not directly comparable. Second, because anti-dengue IgM antibodies do not always remain elevated 2--3 months after infection, especially after a second infection, the serosurvey conducted during December 5--15 likely underestimated the number of recent dengue infections in Brownsville and Matamoros.

Health authorities along the Texas-Tamaulipas border should consider strengthening surveillance for dengue fever, given the potential for future outbreaks with increased risk for DHF. Maintaining active virologic surveillance for circulating serotypes also is important to provide early warning of possible epidemics. Clinicians in the south Texas area and members of the public should be aware of the potential for DHF in addition to dengue fever in the region. Furthermore, clinicians should be trained to recognize and manage DHF. Early recognition and diagnosis of DHF and careful fluid management can reduce the case fatality rate in cases with shock to less than 1%. Public health officials should continue outreach activities to advise communities of prevention measures, including effective mosquito surveillance and reduction programs.

Acknowledgments

This report is based, in part, on contributions from DJ Gubler, Asia-Pacific Institute of Tropical Medicine and Infectious Diseases, Honolulu, Hawaii; J Ramirez, City of Brownsville Public Health Dept, Texas; R Burton, Texas Dept of State Health Svcs; and state and local health departments in Texas and Tamaulipas, Mexico.

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* ≤100,000 platelets/mm^3.

† Boletín Epidemiolgía [Spanish] México, D.F. Dirección General de Epidemiología, 2000--2006. Available at http://www.dgepi.salud.gob.mx/boletin/boletin.htm.

§ Defined as the presence of anti-dengue IgM antibody, dengue viral identification by polymerase chain reaction, or virus isolation from a blood sample of a patient with clinically compatible symptoms.

¶ DHF is classified into four grades of severity; grades III and IV are considered to be dengue shock syndrome. Grade I: Fever accompanied by nonspecific constitutional symptoms; the only hemorrhagic manifestation is a positive tourniquet test and/or easy bruising. Grade II: Spontaneous bleeding in addition to the manifestations of Grade I patients, usually in the forms of skin or other hemorrhages. Grade III: Circulatory failure manifested by a rapid, weak pulse and narrowing of pulse pressure or hypotension, with the presence of cold, clammy skin and restlessness. Grade IV: Profound shock with undetectable blood pressure or pulse (*2*).

Figure 1

FIGURE 1. Jurisdictions affected by dengue fever outbreak — Texas–Mexico border, 2005

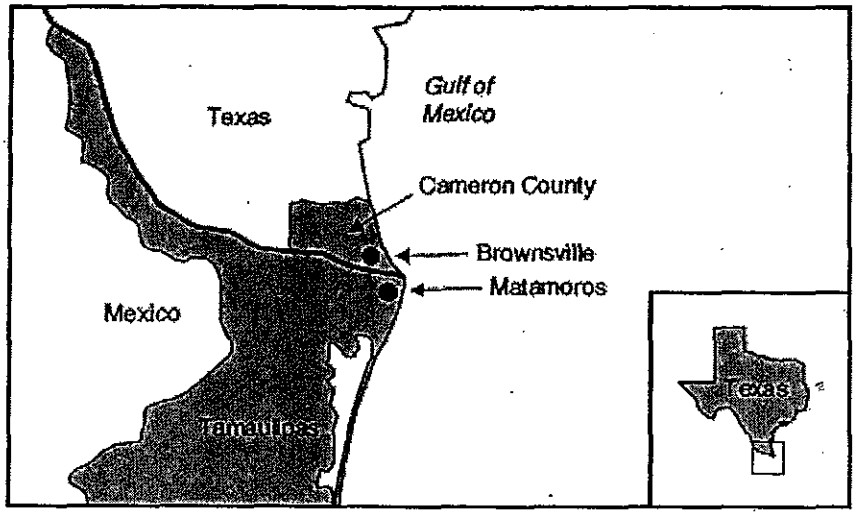

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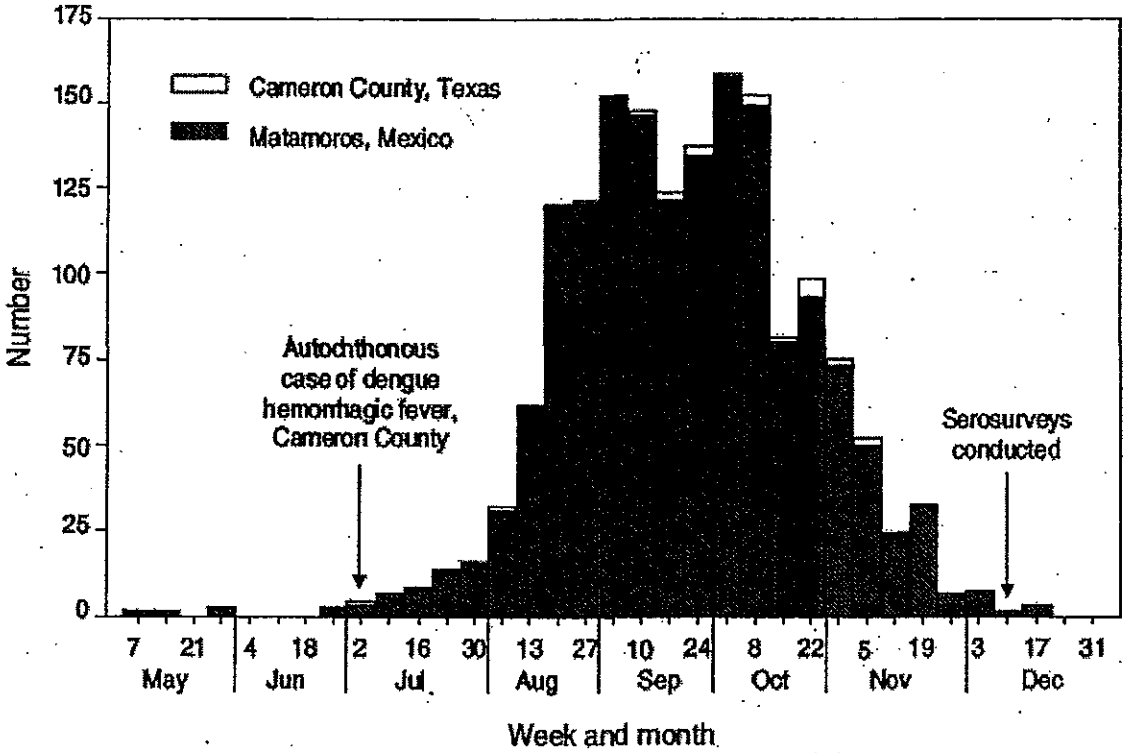
Figure 2

FIGURE 2. Number of cases of dengue fever, by week of report — City of Matamoros, Mexico,* and Cameron County, Texas,† 2005



*n = 1,596.
†n = 25.

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Box

BOX. World Health Organization case definition for dengue hemorrhagic fever

The following must all be present:

- Fever, or history of acute fever, lasting 2–7 days, occasionally biphasic.
- Hemorrhagic tendencies, evidenced by at least one of the following:
  - — a positive tourniquet test;
  - — petechiae, ecchymoses, or purpura;
  - — bleeding from the mucosa, gastrointestinal tract, injection sites, or other locations;
  - — hematemesis or melena.
- Thrombocytopenia ($\leq$100,000 platelets/mm^3).
- Evidence of plasma leakage because of increased vascular permeability, manifested by at least one of the following:
  - — an increase in the hematocrit $\geq$20% above average for age, sex, and population;
  - — a decrease in the hematocrit following volume-replacement treatment $\geq$20% of baseline;
  - — signs of plasma leakage such as pleural effusion, ascites, and hypoproteinemia.

SOURCE: World Health Organization. Dengue haemorrhagic fever: diagnosis, treatment, prevention and control. 2nd ed. Geneva, Switzerland: World Health Organization, 1997. Available at http://www.who.int/csr/resources/publications/dengue/Denguepublication/en.

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医薬品
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一般的名称	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン	研究報告の公表状況	Transactions of the Royal Society of Tropical Medicine and Hygiene 2007; 101(7): 738-739	公表国 日本		
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研究報告の概要

28歳の日本人女性が、2006年6月29日から2006年7月4日までベトナムを、2006年7月4日から10日まで中国を訪問した。7月6日に、彼女は関節痛を伴う高熱を呈した。6日後に、四肢に斑点状丘疹を生じた。彼女は、7月11日に我々のクリニックを訪問した。発熱または出血の徴候が観察されなかったにもかかわらず、臨床検査では血小板減少症（39000個/μL）と白血球減少症（1730の白血球数/μL）を示した。デングウイルス・ゲノムは、7、14または21日目に、血漿または末梢血単核細胞（PBMC）サンプル中には検出されなかった。しかしながら、デングウイルス-1型（DENV-1）ゲノムが、7、8及び14日目の尿サンプル、並びに7日目のだ液サンプルから検出された。尿サンプルからのPCR産物のヌクレオチド配列の分析から、検出されたゲノムはデングウイルス-1のゲノムであることが確認された。
DENV感染は、通常血清または血漿サンプルでELISAによるデング特異的IgM及びRT-PCRによるウイルスゲノムの検出によって確認される。RT-PCR試験は疾患の初期段階のみ検出可能であり、ELISA試験は疾患の初期は陰性であって予想的診断を下すことができるに過ぎない。従って、検査法の組合せが、デングウイルス感染症の確度の高い診断を下すために必要である。尿とだ液サンプルは侵襲的方法を使用せずに集めるのが容易であり、尿はかなりの量を集めることができる。更なる検討が必要であるが、本研究の結果から、尿と唾液のDENVゲノムの検出が、ウイルス性出血の小児にとって有効且つ支援となる代替診断法となり得ることを示唆している。

報告企業の意見	今後の対応
デング熱患者の血漿中ではなく尿及び唾液中からデングウイルスが検出されたとの報告である。 血漿分画製剤からのデングウイルス伝播の事例は報告されていない。万一、原料血漿にデングウイルスが混入しても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体、抗HTLV-I抗体陰性で、かつALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）及びpH3.9～4.4の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

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CASE REPORT

Confirmation of dengue virus infection by detection of dengue virus type 1 genome in urine and saliva but not in plasma

安全
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Y. Mizuno[a,*], A. Kotaki[b], F. Harada[b], S. Tajima[b], I. Kurane[b], T. Takasaki[b]

[a] *Disease Control and Prevention Centre, International Medical Centre of Japan, 1-21-1, Toyama, Shinjuku, Tokyo 162-8655, Japan*
[b] *Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan*

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Dengue hemorrhagic fever;
Diagnosis;
PCR;
Urine;
Saliva

Summary We successfully detected dengue virus (DENV) genome in urine and saliva but not in plasma samples from a Japanese dengue fever patient. The results of the present study suggest that detection of DENV genome in urine and saliva can be an effective diagnostic method, particularly for children with viral hemorrhage.
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1. Case report

A 28-year-old Japanese woman visited Vietnam from June 29 to July 4 2006 and China from 4 to 10 July 2006. On 6 July, she developed a high fever with arthralgia. Six days later, maculopapular rashes appeared on her limbs. She visited our clinic (International Medical Centre of Japan) on 11 July. Although no fever or sign of hemorrhage was observed, laboratory tests showed thrombocytopenia (39 000 platelets/μl) and leukopenia (1730 white blood cells/μl). A diagnosis of dengue fever was considered, and RT-PCR, IgM-capture ELISA and IgG ELISA were carried out (Nawa et al., 2001).

Anti-dengue virus (DENV) IgM and IgG were identified in plasma samples on days 7, 14 and 25 after the onset of symptoms (Table 1). These samples were examined for virus genome by real-time RT-PCR (TaqMan RT-PCR) (Ito et al., 2004). DENV genome was not detected in plasma or peripheral blood mononuclear cells (PBMC) samples on day 7, 14 or 21. However, DENV-type 1 (DENV-1) genome was detected in urine samples on days 7, 8 and 14, and in a saliva sample on day 7.

Nucleotide sequences of PCR products from urine samples were determined with BigDye Terminator version 3.1 (Applied Biosystems, Foster City, CA, USA) and PCR primers. Sequence analysis with a PRISM 3100 Avant Genetic Analyzer (Applied Biosystems) confirmed that the detected genome

* Corresponding author. Tel.: +81 3 3202 7181; fax: +81 3 3202 1012.
E-mail addresses: yamizuno@imcj.hosp.go.jp, mizunomd@hotmail.com (Y. Mizuno).

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Rohwer, Robert presentation at the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) Feb 12, 2004. Recent experimental studies in animals regarding TSE infectivity in blood and transfusion transmission of TSE's - Review of recent experiments in rodents and in sheep.

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研究報告の概要

○感染症最新情報:シャーガス病

AABBは米国疾病対策予防センター(CDC)からAABBシャーガス病バイオビジランス・ネットワークを強化するための資金提供を受けた。AABBによると、「これによって、参加している検査施設と行政双方にとって、利便性とシステムの価値が高まるだろう」とのことである。このネットワーク(電子的疾病サーベイランス報告システム)は、シャーガス病の病原因子である*Trypanosoma cruzi*抗体陽性の供血者のスクリーニングと確認検査を検知する。このプログラムのウェブサイトによると、2007年9月13日時点で710件の供血が繰り返し*T. cruzi*抗体陽性となり、追加のRIPA検査(放射性免疫沈降法)を実施した。このうち196検体がRIPA陽性、486検体が陰性だった。残りの検体については結果保留となっている。13の検査施設がシャーガス・ネットワークにデータを報告した。18施設が報告のために現在アクセスしている。

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
AABBシャーガス病バイオビジランス・ネットワークによると、2007年9月13日時点で710件の供血が繰り返し*T. cruzi*抗体陽性となり、追加のRIPA検査では196検体が陽性、486検体が陰性だったとの報告である。	日本赤十字社は、輸血感染症対策として献血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、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。

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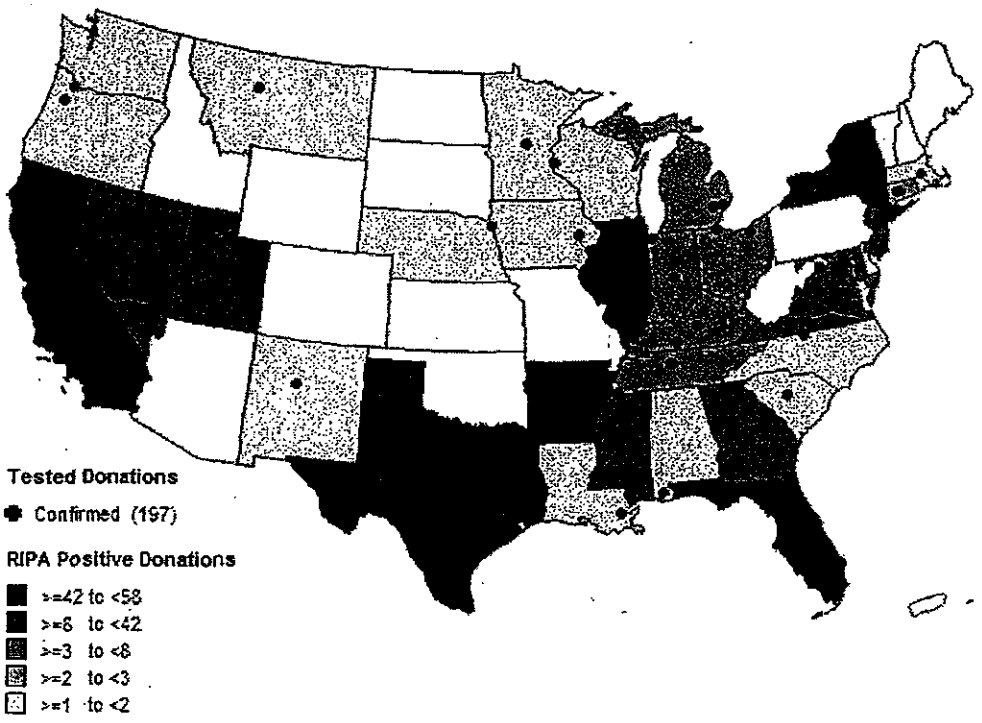
INFECTIOUS DISEASE UPDATES (continued from page 18)

CHAGAS' DISEASE

AABB has received funding from the Centers for Disease Control and Prevention to explore enhancements to the AABB Chagas' Disease Biovigilance Network. According to AABB, "the enhancements would improve usability and value of the system for both participating laboratories and the public." The network – a custom electronic disease surveillance reporting system – tracks screening and confirmatory testing of blood donors with antibodies to *Trypanosoma cruzi*, the agent of Chagas' disease. Source: *AABB Weekly Report*, 9/7/07. According to the program's Web site, as of September 13, 710 repeat reactive donations were tested by the supplemental RIPA test for the antibody to *T. cruzi*, the agent for Chagas' disease. 196 of the repeat reactive donations were RIPA positive; 486 were RIPA non-reactive. Results are pending on the remaining samples. Thirteen testing laboratories reported data into the Chagas Network; eighteen testing laboratories now access the Chagas Network for reporting purposes.



Source: www.aabb.org/Content/Programs_and_Services/Data_Center/Chagas/chagas.htm

INFLUENZA, AVIAN

German health authorities last week ordered the slaughter of more than 200,000 ducks at two farms in Bavaria after tests indicated the presence of the H5N1 strain of bird flu. The head of Bavaria's state office for health and food safety, Volker Hingst, called the slaughter "a purely precautionary measure," taken after "laboratory indications of H5N1" were found. The birds showed no overt signs of the disease, he said. The two farms are located near Schwandorf, east of Nuremberg. Last month, more than 160,000 ducks were slaughtered at another Bavarian poultry farm following an outbreak of the disease. Officials have said that contaminated straw was the likely source in that case. The two new farms with infected ducks are subsidiaries of that farm, authorities said. (Source: Associated Press, 9/7/07) ♦

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 8. 23	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Comeau P. CMAJ. 2007 Jul 31;177(3):242.	公表国 カナダ
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			

研究報告の概要

○カナダ血液サービス、シャーガス病スクリーニング導入へ

カナダ血液サービスは、2008年後半の血液製剤製造プロセス見直しの際に北緯49度以北では稀にしか見られないシャーガス病のスクリーニングを開始する予定である。

Dana Devine副社長によると、2種類のシャーガス病検査法がカナダ保健省の認可を待っている状態である。供血血液の検査実施は、血小板製剤の製造を「バフィーコート」法に切替え、細菌検査を導入する時期以降になる予定である。

メキシコや中南米の住民800万人～1,100万人がシャーガス病の保因者であり、毎年45,000人以上の人が死亡している。原虫*Trypanosoma cruzi*が引き起こす感染症で、主に農村部の貧しい地域で昆虫が媒介する。一部の感染者には症状がないが、発症した場合は発熱、むくみ、脾臓や肝臓、リンパ節の肥大、心臓の炎症などを、感染直後や、時には感染数十年後に引き起こす。

媒介昆虫は、睡眠中の人の顔を噛む。原虫は媒介昆虫の排泄物から、人の粘膜や皮膚の傷口を通って感染する。汚染された生の食物による感染、母子感染、臓器移植や実験室暴露による感染もある。

カナダでは、これまでに輸血による感染が2例マニトバ州で発生した(1986年及び2000年)。患者は原疾患で死亡している。ワシントン州及びカリフォルニア州の高リスク集団を対象にした研究では、0.2%が感染しており、そのうち20%が他人に感染させる危険があることが判明した。Devineによると、カナダの潜在的シャーガス病患者は感染地域の訪問歴や居住歴を尋ねる問診によって供血者から排除されてきた。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
カナダ血液サービスは、2008年後半にシャーガス病のスクリーニングを開始する予定であるとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。今後も引き続き情報の収集に努める。

JRC2007T-056

Lancet publisher to sell arms business

Responding to pressure from physicians, readers and leading medical journals, the company that publishes *The Lancet* will stop hosting trade shows that promote arms and the defence industry (*CMAJ* 2007;176[10]:1265).

Reed Elsevier will begin withdrawing from its exhibitions, conferences, congresses and meetings in the second half of this year, CEO Sir Crispin Davis said in a June 1 news release.

"Our defence shows are quality businesses which have performed well in recent years," Davis said. "Nonetheless, it has become increasingly clear that growing numbers of important customers and authors have very real concerns about our involvement in the defence exhibitions business."

In March, more than 50 editorial consultants at *The Lancet* posted a letter at cmaj.ca arguing that Reed Elsevier's involvement in the arms trade was incompatible with the journal's commitment to health. That was followed by an online petition which contained nearly 1000 signatures, and calls by *The Lancet*, *BMJ*, *the Journal of the Royal Society of Medicine*, the London School of Hygiene and Tropical Medicine, and by *CMAJ* Editor-in-Chief Paul Hébert asking Reed Elsevier to stop participating in the arms' trade.

"We have listened closely to these concerns and this has led us to conclude that the defence shows are no longer compatible with Reed Elsevier's position as a leading publisher of scientific, medical, legal and business content," Davis said in the news release.

Pressure from *The Lancet* and *BMJ*, and the public Campaign Against Arms Trade, was critical in influencing Reed Elsevier, says Anna Jones, the campaign coordinator.

"It was the concerted efforts of Reed's stakeholders, and particularly healthcare professionals, which brought the pressure of that concern to bear," she wrote to *CMAJ* in an email. "The voices of readers and contributors to Reed's publications ... were extremely important in persuading Reed Elsevier that continuing to take part in the arms trade was compromising their credibility within the scientific, medical, legal and business communities."

In a related issue, members of Physicians for Global Survival have written the CMA asking MD Management to create an ethical investment portfolio for physicians who do not wish their money to support the arms and defence industries.

Despite Reed Elsevier's decision, MD Management is not planning further restrictions on its funds, says Guy Bélanger, president and CEO of MD Funds Management. MD funds currently do not hold any tobacco company stock.

"We haven't really received any significant requests from clients generally to increase the number of restrictions on our portfolio," Bélanger told *CMAJ*, adding that if individual physicians do request ethical investments, the fund managers can find them.

Although MD Management has received "the odd letter" requesting that it divest from arms manufacturers or the defence industry, "we also receive the [odd] letter on the other side of the equation, which makes it difficult to manage within a fund," Bélanger said.
— Laura Eggertson, Ottawa

DOI:10.1503/cmaj.070884

Canadian Blood Services to screen for Chagas disease

Canadian Blood Services will commence testing for a tropical disease rarely found north of the 49th parallel when the blood donation production process is overhauled in mid-to-late 2008.

Dana Devine, Canadian Blood Services vice-president of medical, scientific and research affairs, says 2 tests for Chagas disease (also called American trypanosomiasis) are in line for Health Canada approval. But testing donated blood for Chagas will be delayed until the agency switches to the "Buffy Coat" method of blood platelet production. (Used in Europe for 2 decades, the method produces a more consistent product and yields more platelets. Buffy Coat refers to the layer of white blood cells left atop the mass of red cells when whole blood is centrifuged). "This is a production method that allows us to prepare a pool platelet product for transmission that we can test for bacteria," says Devine.

Between 8 and 11 million people in Mexico, Central America, and South America are believed to have Chagas, with upwards of 45 000 deaths a year. Caused by *Trypanosoma cruzi* parasites, it is transmitted by insects found mainly in rural, poverty-stricken areas. While some victims are symptom free, others suffer fever, swelling, an enlarged spleen, liver and lymph nodes, and heart inflammation immediately after infection or sometimes decades later (*CMAJ* 2006; 174[8]:1096).

Insects bite sleeping victims, usually on the face (earning them the moniker "kissing bugs"). The parasites, carried in feces, infect through mucous membranes or breaks in skin. Infection also occurs through consumption of contaminated uncooked food, congenital transmission, organ transplants and accidental laboratory exposure.

Two cases are known to have been transmitted via blood transfusions in Canada, both in Manitoba (1986 and 2000). The patients died of underlying conditions. Studies of high-risk populations in Washington and California showed 1/500 may have been infected, with 20% of those able to transmit the infection. Devine says potential Chagas sufferers in Canada have been screened out for years via an agency questionnaire that asks if potential donors have visited or lived in affected areas.

Is waiting until 2008 to test too long?

No, says Dr. Jay Keystone, Toronto General Hospital's Tropical Disease Unit staff physician. "There has always been a low but definite risk to the blood supply from people from the Chagas areas of the world." But while it's prudent to test, suggesting Chagas is a medical emergency "takes it all out of perspective," he adds. — Pauline Comeau, Ottawa

DOI:10.1503/cmaj.070882

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 2007年9月10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	乾燥 pH4 処理人免疫グロブリン		研究報告の公表状況	Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation Journal of general virology (England) Aug 2007, 88 (Pt 8) p2162-7	公表国 英国	
販売名（企業名）	サングロボール（CSL ベーリング株式会社）					

研究報告の概要

問題点（PARV4 の PARV5 の DNA 配列を解析比較）

パルボウイルスは小型でエンベロープを持たない直鎖一本鎖 DNA ウイルスである。PARV4 は薬物使用者の血漿から PCR 法で最近同定された変異型のヒトパルボウイルスである。また血液製剤や血漿分画製剤の製造に用いるプール血漿中で発見されている。しかしヒトの疾病への関与は判明してない。

筆者らは製造用のプール血漿から分離された 4 種の PARV4 ゲノムと 2 種の PARV5 ゲノムを、GenBank のオリジナル PARV4 と DNA 配列を解析して比較した。

PARV4 の ORF は、オリジナル PARV4 との相同性は約 98-100%で、PARV5 の ORF の相同性は 91-94％であった。

このことから PARV4 には 2 つのサブグループが存在することを示唆している。

筆者らはジェノタイプ 1 とジェノタイプ 2（従来は PARV5 と命名されていた）から構成される PARV4 の単独のウイルス名を使用することを提案している。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
現在はPARV4について詳細が不明であり、検査方法が確立されていないので、今後も情報を収集することに努める。	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。

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ABJ20072203

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Short Communication

Analysis of two human parvovirus PARV4 genotypes identified in human plasma for fractionation

Jacqueline F. Fryer,[1] Eric Delwart,[2,3] Flavien Bernardin,[2,3] Philip W. Tuke,[4] Vladimir V. Lukashov[5,6] and Sally A. Baylis[1]

Correspondence
Sally A. Baylis
sbaylis@nibsc.ac.uk

[1]Division of Virology, National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, EN6 3QG, UK

[2]Blood Systems Research Institute, San Francisco, CA 94118, USA

[3]University of California, San Francisco, CA 94118, USA

[4]Department of Virology, University College London Hospital, The Windeyer Building, 46 Cleveland Street, London W1T 4JF, UK

[5]Laboratory of Experimental Virology, Department of Medical Microbiology, Center for Infection and Immunity Amsterdam (CINIMA), Academic Medical Center, University of Amsterdam, 1105AZ Amsterdam, The Netherlands

[6]Laboratory of Immunochemistry, D. I. Ivanovsky Institute of Virology, 123098 Moscow, Russia

The presence of the novel parvovirus PARV4 and a related variant, PARV5, was recently demonstrated in pooled plasma used in the manufacture of blood and plasma-derived medicinal products. DNA sequence analysis of nearly full-length genomes of four PARV4 and two PARV5 strains from manufacturing plasma pools is now presented. Like PARV4, PARV5 encodes two non-overlapping open reading frames (ORF1 and ORF2), homologous to the non-structural and capsid proteins of other parvoviruses, respectively. A highly conserved region in ORF2 contains phospholipase A_2 motifs involved in parvovirus infectivity. Hybridization of strand-specific probes to DNA extracted from high-titre, PARV4-positive plasma revealed that the positive and negative strands are packaged into PARV4 virions in similar quantities. This extended analysis of nearly full-length PARV4 and PARV5 sequences suggests that they are closely related genotypes and the use of a single virus name, PARV4, comprising genotypes 1 and 2 (previously termed PARV5) is proposed.

Received 4 October 2006
Accepted 30 April 2007

Parvoviruses are small, non-enveloped viruses containing linear, single-stranded DNA genomes. They infect a diverse range of vertebrate and invertebrate hosts. PARV4 is a novel human parvovirus recently identified in plasma from an intravenous drug user (IVDU) by using a sequence-independent PCR amplification method (Jones *et al.*, 2005). This individual presented with symptoms of acute viral infection, including fatigue, vomiting, diarrhoea, sore throat, neck stiffness and joint pains, and was also co-infected with hepatitis B virus. Nothing is yet known about the role of PARV4 in human disease. DNA sequence analysis of the PARV4 genome identified two open reading frames, ORF1 and ORF2, with significant similarity to the non-structural and capsid proteins of parvoviruses, respectively. Phylogenetic analysis showed that PARV4 was distinct from other known animal or human parvoviruses (Jones *et al.*, 2005).

The prototype human parvovirus B19 (B19V) is a frequent contaminant of plasma pools used in the manufacture of plasma-derived medicinal products (reviewed by Brown *et al.*, 2001; Laub & Strengers, 2002). PARV4, and a related variant virus termed PARV5, have been identified in approximately 4–5 % of these manufacturing pools (Fryer *et al.*, 2006, 2007b). PARV4 and PARV5 were found to share 92 % nucleotide identity over a 178 bp region of ORF1. In the present study, more extensive sequence analysis has been performed on PARV4 and PARV5 strains identified in recent and older manufacturing plasma pool samples (Fryer *et al.*, 2006, 2007b). Strand-specific probes were used to analyse the packaging of the PARV4 genome.

Nucleic acid was extracted from 1 ml volumes of manufacturing plasma pools, sourced from Europe and North America, as described previously (Baylis *et al.*, 2004).

The GenBank/EMBL/DDBJ accession numbers for the PARV4 and PARV5 sequences determined in this study are DQ873386–DQ873391.

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Samples were screened for the presence of PARV4 and PARV5 DNA by using primers to ORF1 and/or ORF2, as described previously (Fryer *et al.*, 2006, 2007b), and nearly full-length PARV4 and PARV5 sequences determined in positive plasma pools. To avoid sequencing on different templates, two approximately 2.5–2.8 kb overlapping cDNA fragments spanning the ORF1 and ORF2 coding regions were amplified from extracted plasma samples. The 5′ PARV4/5 fragment (nt 142–2977, GenBank accession no. AY622943) was amplified by using primers PARV4/5seq1 (5′-CGGTCCCGCGAAAATTACGTATT-3′) and PVORF2R (Fryer *et al.*, 2007b), whilst the 3′ fragment (nt 2710–5247 of GenBank accession no. AY622943) was amplified by using primers PVORF2F (Fryer *et al.*, 2007b) and PARV4/5seq21 (5′-CGCGAAAATTGCGTATTTCCG-CT-3′). The reaction mixture consisted of 1 × Phusion HF Buffer (Finnzymes OY), 200 μmol each dNTP l^{-1}, 10 pmol each primer, 1 U proofreading Phusion Hot Start DNA Polymerase (Finnzymes OY) and 5 μl extracted DNA in a final volume of 50 μl. Thermal cycling was performed as follows: 98 °C for 10 s, followed by 45 cycles of 98 °C for 10 s, 63 °C for 30 s and 72 °C for 3 min. PCR products were purified, cloned into the pT7 Blue vector (Novagen) and both strands were sequenced as described previously (Fryer *et al.*, 2006). Sequences were assembled and ORFs were mapped by using the GCG software package, version 10.2 (University of Wisconsin). Comparisons of deduced protein sequences were performed by using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Phylogenetic analysis of nearly full-length PARV4 and PARV5 genomes was performed alongside full genome sequences of other members of the subfamily *Parvovirinae* (Lukashov & Goudsmit, 2001). Sequences were aligned by using CLUSTAL_W (Chenna *et al.*, 2003), and a neighbour-joining tree (nucleotide distance with Jukes–Cantor correction, pairwise gap deletion) with bootstrap resampling (100 replicates) was constructed by using MEGA3 software (Kumar *et al.*, 2004). Bootscan analysis was performed by using the SimPlot software, version 3.5.1 (Lole *et al.*, 1999).

For analysis of the encapsidation pattern of PARV4 virions, DNA was extracted from a high-titre PARV4-positive individual plasma donation and negative plasma controls as described above. This plasma sample was also positive for hepatitis C virus (HCV) RNA and was identified by a routine screening process to eliminate HCV-positive donations prior to the pooling of plasma for fractionation. Positive and negative plasmid controls were also tested. The PARV4-positive plasmid control contained a 1683 bp insert of PARV4 ORF2 (nt 1705–3387, GenBank accession no. AY622943). The PARV4-negative plasmid control contained a 1471 bp insert of PARV4 ORF1 (nt 603–2073 of AY622943). The PARV4 fragments were cloned into the vector pT7 Blue (Novagen) as described previously (Fryer *et al.*, 2006). All DNA samples were denatured with 0.25 M NaOH at 37 °C for 15 min prior to spotting onto a Hybond XL membrane (GE Healthcare). Nucleic acids were UV cross-linked. Membranes were pre-hybridized with Rapid-hyb buffer (GE Healthcare) in accordance with the manufacturer's instructions. Strand-specific probes were prepared by 5′-end labelling of positive- and negative-sense oligonucleotides in ORF2 of PARV4 (PVORF2F and PVORF2R; Fryer *et al.*, 2007b) by using [γ-^{32}P]ATP and T4 polynucleotide kinase (TaKaRa) and used in accordance with the manufacturer's instructions. Replicate filters were hybridized at 42 °C with either the positive- or negative-sense probes. Following hybridization, filters were washed at 42 °C with 0.1 × SSC, 0.1 % SDS, in accordance with the manufacturer's instructions (GE Healthcare). Blots were quantified by using the InstantImager (Perkin Elmer) electronic autoradiography system.

Nearly full-length sequences (GenBank accession numbers indicated), comprising ORF1 and ORF2, were determined for the following strains of PARV4: BR10749-4 (DQ873386), BR11955-4 (DQ873388), A23-4 (DQ873389) and C51-4 (DQ873387); and for PARV5, BR10627-5 (DQ873390) and C25-5 (DQ873391). Strains BR10749-4 and BR10627-5 were identified in our preliminary study of manufacturing plasma pools (Fryer *et al.*, 2006), whilst the other strains were identified in further screening studies of plasma pools (Fryer *et al.*, 2007b). Strains BR10749-4, BR11955-4 and BR10627-5 were from plasma samples obtained between 2004 and 2005, whereas A23-4, C51-4 and C25-5 date from 1990–1993.

As with B19V, PARV4 and PARV5 genomes comprise two main ORFs: ORF1 (1992 nt, encoding 664 aa) and ORF2 (2745 nt, encoding 915 aa). However, unlike B19V, the two ORFs do not overlap and are separated by 103 and 106 nt for PARV4 and PARV5, respectively. By BLASTP analysis, PARV4 and PARV5 ORF1-encoded proteins are homologous to the non-structural protein of parvoviruses, NS1, showing greatest amino acid identity with NS1 of hamster (H-1) and goose parvoviruses (50 and 43 % identity, respectively). The amino acid sequence contains parvovirus-conserved motifs associated with rolling-circle replication [xuHuHuuux (aa 97–99) and uxxYuxxKxx (aa 157–161); Ding *et al.*, 2002] and ATPase [A site GxxxxGK(T/S) (aa 334–341) and B site uuuu(D/E)(D/E) (aa 374–379); Astell *et al.*, 1987; Ding *et al.*, 2002]. The ORF2-encoded proteins are homologous to the viral capsid protein of parvoviruses, VP1, sharing approximately 45 and 41 % amino acid identity with VP1 of hamster parvovirus (H-1) and B19V-Au genotype 1, respectively. The amino acid sequence contains phospholipase A_2 motifs [YxGxG (aa 224–228) and HDxxY (aa 247–251], required for parvovirus infectivity (Zádori *et al.*, 2001). These motifs are completely conserved between PARV4 and PARV5.

Pairwise comparisons of each ORF of PARV4 and PARV5 strains indicate that ORF2 is more conserved than ORF1 (Table 1), with most nucleotide differences limited to synonymous substitutions (91 % of nucleotide substitutions in ORF2 are synonymous, compared with 89 % in ORF1). Consequently, the amino acid sequences are more conserved, particularly for ORF2, suggesting that if PARV4 and PARV5 represent two distinct genotypes, they might

Table 1. Nucleotide and amino acid identities between the original PARV4 strain (GenBank accession no. AY622943) and PARV4 and PARV5 strains from plasma pools

Virus/strain	Identity (%) (nucleotide/amino acid)	
	ORF1	ORF2
PARV4*		
BR10749-4†	99.7/99.5	99.9/100.0
C51-4‡	99.6/99.5	99.9/99.9
BR11955-4†	98.2/98.6	98.5/99.6
A23-4‡	98.2/99.4	98.5/99.6
PARV5		
BR10627-5†	90.9/96.8	93.5/98.7
C25-5‡	90.9/97.6	93.4/99.2

*Strains BR10749-4 and C51-4 fall within the same subgroup as the prototype PARV4 strain; strains BR11955-4 and A23-4 represent a separate subgroup of PARV4.
†Strains derived from recent plasma pools (2004–2005).
‡Strains derived from plasma pools from 1990–1993.

be expected to represent a single serotype. This would be analogous to B19V genotypes 1–3, where serological cross-reactivity has been demonstrated *in vitro* by using clinical sera against a genotype 2 virus isolate (Blümel *et al.*, 2005) and baculovirus-expressed genotype 3 virus capsid antigens (Parsyan *et al.*, 2006). PARV4 sequences share approximately 98–100% nucleotide identity with the original PARV4 isolate (GenBank accession no. AY622943), whilst PARV5 sequences share approximately 91–94% nucleotide identity with the original PARV4 sequence (Table 1). Sequence analysis indicates that there are two subgroups of PARV4, with a nucleotide divergence of 2% between strains BR11955-4 and A23-4, and the prototype PARV4 sequence (GenBank accession no. AY622943). Overall, PARV4 and PARV5 strains share approximately 92% nucleotide identity, similar to the level observed between B19V genotypes 1–3 (Servant *et al.*, 2002; Gallinella *et al.*, 2003). Comparison of recent with archived strains within both PARV4 subgroups and PARV5 shows little evidence for evolutionary change over 10–15 years (99.8% nucleotide identity over an approx. 4800–5000 bp region between recent and archived strains). This is in contrast to current evidence suggesting that carnivore and B19V parvoviruses exhibit a high rate of genetic change (Shackelton *et al.*, 2005; Shackelton & Holmes, 2006).

Although not based on full-length genomes, the current sequence analysis of PARV4 and PARV5 strains indicates that the two ends of the viral DNA are related by inverted terminal repeats (ITRs), as is the case for many parvoviruses. In the prototype PARV4 sequence (Jones *et al.*, 2005; GenBank accession no. AY622943), nt 128–244 and 5151–5267 respectively correspond to incomplete 5′ and 3′ ITRs, possibly forming part of the stem structure of the hairpins, with nt 1–128 at the 5′ end forming part of a loop structure (an axis of symmetry lies between nt 6 and 7). Incomplete ITRs obtained in this study are distinct between PARV4 and PARV5 (97–98% nucleotide identity) and are slightly shorter than those of the original PARV4 sequence (GenBank accession no. AY622943), reflecting the length of the sequences obtained. Further sequencing of the ITRs has proved difficult, possibly due to the presence of repeated heptanucleotide sequences (CTTCCGG) identified in the hairpin stems of the original PARV4 sequence (GenBank accession no. AY622943), which are similar to those observed in the ITRs of goose and muscovy duck parvoviruses (Zádori *et al.*, 1995).

Phylogenetic analysis of PARV4 and PARV5 with other members of the subfamily *Parvovirinae* indicates that they are equidistant from human/primate autonomous parvoviruses and the adeno-associated (AAV)/avian viruses (Fig. 1a). Based upon this nearly full-genome analysis, PARV4 and PARV5 are related most closely to porcine parvovirus 2 (PPV-2), a virus identified in swine sera from Myanmar (Hijikata *et al.*, 2001). Recombination analysis of the PARV4, PARV5 and PPV2 sequences indicates that these viruses do not appear to be recombinants of other known viruses (data not shown). Similarly, there was no evidence for recombination between PARV4 and PARV5, with the clustering pattern of all PARV4 and PARV5 strains being the same regardless of whether full-length sequences or individual ORFs were analysed. At the protein level, however, ORFs 1 and 2 of PARV4 and PARV5 showed closest amino acid identity to the non-structural and capsid proteins of hamster parvovirus H-1, a virus causing significant morbidity and mortality in neonatal hamsters (Besselsen *et al.*, 1999). Phylogenetic analysis again indicates that there are two subgroups of PARV4 (also indicated in Table 1); strains BR10749-4 and C51-4 cluster with the original PARV4 sequence (GenBank accession no. AY622943), whereas BR11955-4 and A23-4 represent a different subgroup. Phylogenetic analysis of non-synonymous substitutions also demonstrated that PARV4 and PARV5 sequences formed two separate clusters with bootstrap values of 98–100% (data not shown). Nucleotide similarity plots along nearly full-length PARV4 and PARV5 sequences revealed a highly conserved region (>99% nucleotide identity) between nt 2955 and 3420 (GenBank accession no. AY622943), located at the 5′ end of ORF2 (Fig. 1b). The phospholipase A_2 motifs, required for parvovirus infectivity, are located here. Based upon PARV4 and PARV5 sequence alignments of this region, we have designed a consensus real-time TaqMan PCR to quantify both PARV4 and PARV5 sequences (Fryer *et al.*, 2007b).

The encapsidation pattern of PARV4 was investigated by using strand-specific oligonucleotide probes to ORF2 and nucleic acid extracted from a high-titre PARV4-positive individual plasma donation. Both positive- and negative-sense probes hybridized to the PARV4-positive plasma DNA (sample C, Fig. 2) and PARV4 ORF2 control plasmid DNA (Fig. 2, plasmid concentrations indicated), but not to

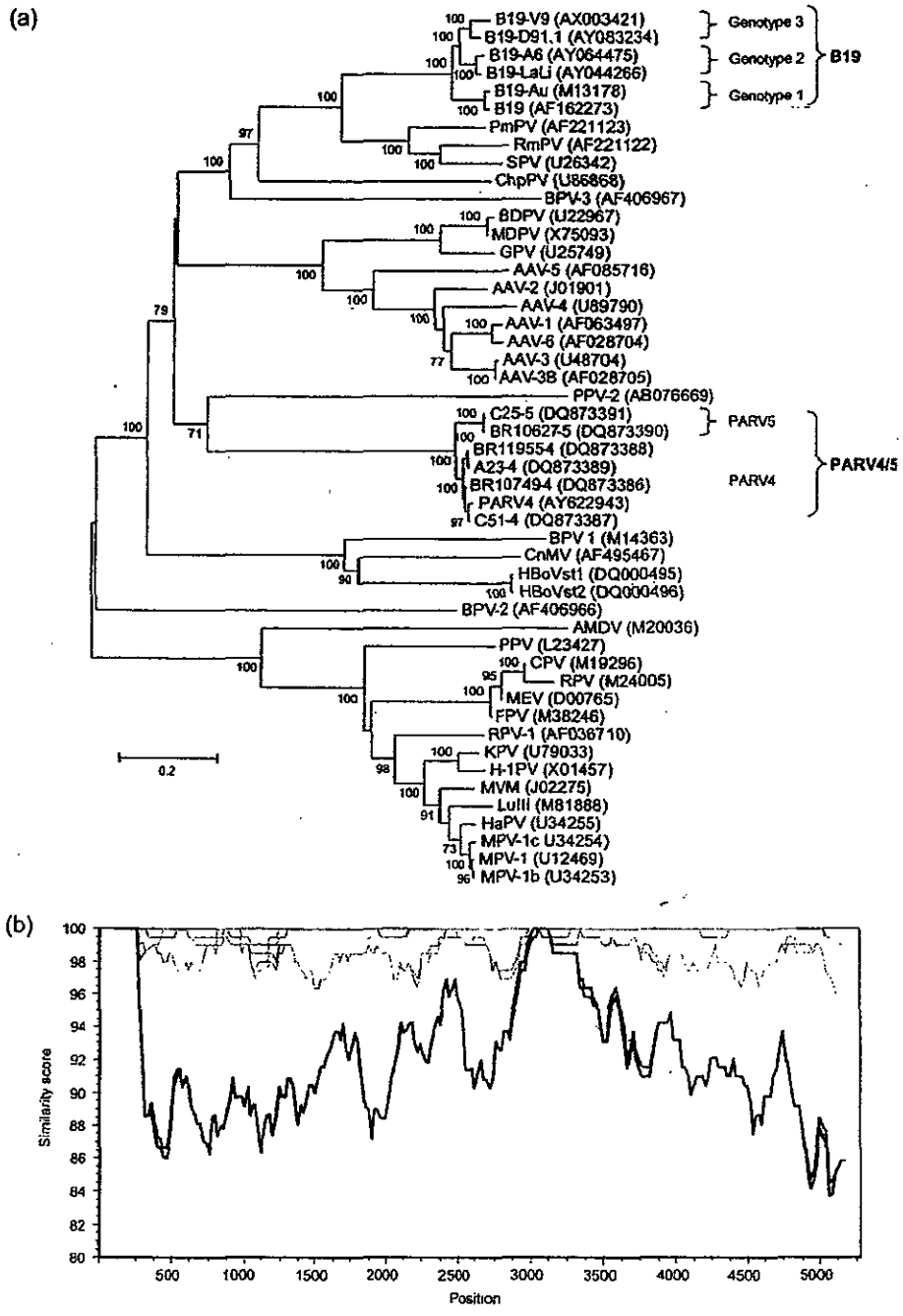

Fig. 1. (a) Phylogenetic analysis of nearly full-length PARV4 and PARV5 sequences (indicated) and other members of the subfamily *Parvovirinae*, including recently sequenced members porcine parvovirus 2 (PPV2) (Hijikata *et al.*, 2001) and human bocavirus (HBoVst1 and HBoVst2) (Allander *et al.*, 2005). B19V genotypes 1 (Shade *et al.*, 1986), 2 [A6 and LaLi (Nguyen *et al.*, 2002; Hokynar *et al.*, 2002)] and 3 [V9 and D91.1 (Nguyen *et al.*, 1999; Servant *et al.*, 2002)] are indicated. Virus names are given according to the ICTV nomenclature (Tattersall *et al.*, 2005), with GenBank accession numbers in parentheses. (b) Graphic representation of nucleotide similarity along nearly full-length sequences of PARV4 (grey lines) and PARV5 (black lines) strains, compared with the prototype PARV4 sequence (GenBank accession no. AY622943), using SimPlot. The co-ordinates for ORF1 and ORF2 are 283–2274 and 2378–5122 nt, respectively. Window, 200 bp; step, 20 bp; GapStrip, on; Kimura (two-parameter); T/t, 2.0.

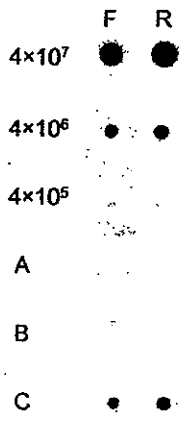

Fig. 2. Analysis of the packaging of the PARV4 genome. DNA from a high-titre PARV4-positive plasma sample was extracted, denatured, immobilized on a nylon membrane and probed with labelled, strand-specific oligonucleotide probes (F, forward; R, reverse) (C). DNA from PARV4-negative plasma (A, B) and PARV4 plasmid controls at 4×10^7, 4×10^6 and 4×10^5 copies per sample are shown.

PARV4 ORF1 control plasmid DNA (data not shown) or to human genomic DNA present in the plasma pools, even when levels were of the order of 10^6 copies ml^{-1} (sample B, Fig. 2). When the number of counts binding to the PARV4-positive plasma DNA sample was determined and compared with the respective plasmid control samples, it was found that the positive and negative strands were present in similar amounts. This packaging pattern is not characteristic of all parvovirus genera (Cotmore & Tattersall, 2006).

The availability of additional, nearly full-length sequences for PARV4 and the related variant PARV5 will assist in further phylogenetic studies and in the design of consensus and discriminatory assays. The recent finding that there are three distinct genotypes of B19V has led to a review of assays available for the detection and quantification of this virus (Baylis *et al.*, 2004, 2007; Hokynar *et al.*, 2004). In some cases, assays fail to detect the newly identified genotypes 2 and 3 of B19V, with consequent issues in terms of clinical diagnosis and viral load determinations in the plasma-fractionation setting, where it is a regulatory requirement to limit levels of B19V (Baylis *et al.*, 2004). The ability to design assays for PARV4 and PARV5 will assist in future studies to determine the role of these viruses in human disease. Nothing is yet known of the pathology associated with these viruses or whether PARV4 and PARV5 cause subclinical infections. However, PARV4 and PARV5 have been identified in plasma from healthy blood donors (Fryer *et al.*, 2006, 2007b) with an increased incidence in febrile patients, including IVDUs and homosexual men (Fryer *et al.*, 2007b), and in HCV-infected individuals (including IVDUs) (Fryer *et al.*, 2007a). Phylogenetic analysis performed in this study shows that PARV4 and PARV5 cluster separately from each other, and could thus be considered as two genotypes of the same virus. We therefore propose the use of a single virus name, PARV4, comprising genotypes 1 and 2 (previously termed PARV5).

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医薬品 研究報告 調査報告書

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				2007. 7. 20	該当なし	

一般的名称	(製造承認書に記載なし)	研究報告の公表状況	ABC Newsletter. 2007 Jul 6.	公表国
販売名(企業名)	合成血「日赤」(日本赤十字社) 照射合成血「日赤」(日本赤十字社) 合成血-LR「日赤」(日本赤十字社) 照射合成血-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○感染症最新情報:マラリア
FDAは、初めて認証された米国のマラリア用迅速テスト、Binax NOWマラリア検査の試用を許可した。「マラリアの標準的臨床検査法は、顕微鏡下で血液検体中の寄生虫を特定しなければならず、訓練と経験を要する難しい作業である」とFDAはニュースリリースの中で述べている。「Binax NOW検査は、非常に迅速で使用が簡便である。」全血検体をディップスティックに数滴つけて15分後には結果が得られる。検査結果の確定には、これからもなお標準的顕微鏡検査法を用いなければならない。「米国ではマラリアはまれであるため、医師及び研究所職員は当疾患の診断に慣れていない。Binax NOW検査は、他の臨床検査法と併用した場合、米国でマラリアをより速やかに診断するのに役立つ新たなツールとなる。」とFDA医療機器・放射線保健センターのDaniel Schultz医師は言った。米国外のマラリア流行地域で行った多施設試験において、標準的顕微鏡診断と比較して当該検査の正確度は95%であった。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
FDAは、初めて認証された米国のマラリア用迅速テスト、Binax NOWマラリア検査の試用を許可したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

2008 Yellow Book Available in Print and on the CDC Web site

Mexican risk areas for malaria revised

The Centers for Disease Control and Prevention has issued the 2008 edition of Health Information for International Travel (long called "The Yellow Book" for its bright yellow cover). Published every two years as a reference for those who advise international travelers of health risks, the Yellow Book and a related Web site are widely used by the nation's blood centers to identify areas around the globe that CDC considers endemic for malaria.

Revised risk information for malaria includes the following for Mexico: "Risk is limited to areas infrequently visited by travelers including small foci along the Guatemala and Belize borders in the states of Chiapas, Quintana Roo, and Tabasco; rural areas in the states of Nayarit, Oaxaca, Sinaloa; and in an area between 24°N and 28°N latitude, and 106°W and 110°W longitude, which lies in parts of Sonora, Chihuahua, and Durango. No malaria risk exists along the United States-Mexico border. No malaria risk exists in the major resorts along the Pacific and Gulf coasts."

The Yellow Book home page is: wwwn.cdc.gov/travel/contentYellowBook.aspx

INFECTIOUS DISEASE UPDATES

vCJD

Cambridge-based ProMetic BioSciences Ltd (PBL) has announced a $1.7 million deal to develop its proprietary prion-binding ligands, with a prominent European plasma fractionator. The ligand technology will be used to minimize the risk of transmission of variant Creutzfeldt-Jakob Disease (vCJD), the human form of "mad cow disease," by plasma-derived products. The technology, developed by Pathogen Removal and Diagnostic Technologies (PRDT), a collaboration with the American Red Cross, is the prion capture element of the P-Capt prion reduction filter used for the treatment of red blood cell concentrate. It will be utilized in a program to bind and remove any abnormal prion protein – PrPsc, the agent responsible for vCJD – that might be present in donor plasma. "Although the risk of cross infection is relatively low after the establishment of treatment protocols, the filter provides an extra level of safety due to the absence of a commercially available diagnostic test for detection of the blood-borne form of the vCJD agent," ProMetic said. Prometic President & CEO Pierre Laurin said: "The efficacy of PRDT's technology in combating the risk of transmission of vCJD in transfusion blood is already established and the P-Capt filter is approved for treatment of red blood cell concentrate. Its application to increase the safety of plasma and plasma derivatives is an obvious extension of the use of this technology." As part of the program, PBL will manufacture and validate an affinity capture material comprising the PRDT ligand, attached to synthetic resin particles. "While for competitive reasons we are not disclosing the name of PBL's partner in this agreement, we are delighted to open a significant new market opportunity for PRDT's technology," Mr. Laurin said. (Source: Business Weekly (UK), 7/4/07)

MALARIA

The Food and Drug Administration has cleared for laboratory use the first authorized US rapid test for malaria – the Binax NOW Malaria Test. "Standard laboratory tests for malaria require identifying parasites in a blood sample under a microscope, a difficult task that requires training and experience," FDA said in a news release. "The Binax NOW test is significantly faster and easier to use." Results are available in 15 minutes after a few drops of whole blood are placed on a dipstick. The test parasites. Results still must be confirmed using

(continued on page 20)

INFECTIOUS DISEASE UPDATES (continued from page 19)

standard microscopic evaluation. "Since malaria is uncommon in the US, clinicians and lab personnel may not be accustomed to diagnosing this disease," said Daniel Schultz, MD, director of FDA's Center for Devices and Radiological Health. "When used in combination with other laboratory tests, the Binax NOW test provides an additional tool to help them diagnose this disease faster in the United States." The assay was 95 percent accurate compared with standard microscopic diagnosis in a multi-center study outside the United States in areas where malaria is prevalent. The test is manufactured by Binax Inc., a subsidiary of Inverness Medical Innovations Inc. of Scarborough, Maine. (Source: FDA News, 6/26/07)

SARS

An international team of investigators has identified the first human antibodies that can neutralize different strains of the virus responsible for outbreaks of severe acute respiratory syndrome (SARS). The researchers, from the National Institutes of Health and elsewhere, used a mouse model and *in vitro* assays to test the neutralizing activity of the antibodies. Their findings appear in the July 2, 2007, early online edition of the *Proceedings of the National Academy of Sciences*. The study is important because it is unlikely that the viral strain that caused the outbreak in people in 2002 still exists in nature. "What we need to prove for any vaccine, therapeutic, antibody, or drug is that it is effective not only against the strain of SARS virus isolated from people, but also against a variety of animal strains, because animals will be a likely source for re-emergence of the SARS virus," the researchers said. The discovery of two effective antibodies has the advantage that a newly emergent variation of the SARS coronavirus might be insensitive to neutralization with one, but still susceptible to the other. "Our results demonstrate novel potential antibody-based therapeutics against SARS that could be used alone or in combination ... these human antibodies could be also used for diagnosis and as research reagents in the development of vaccines and inhibitors," the authors said. **Citation:** Zhu Z *et al.* Potent cross-reactive neutralization of SARS coronavirus isolates by human monoclonal antibodies. Proc Natl Acad Sci USA 2007;104, No. 27 [E-pub July 3, 2007]

WEST NILE VIRUS

A Winnipeg, Manitoba-area blood donor is the first human case of West Nile virus (WNV) this year in Canada. The individual tested positive by mini-pool NAT after a June 19 blood donation at Canadian Blood Services and tested positive by mini-pool NAT. Initial reports indicate that the individual now has mild symptoms. Further investigation is underway to confirm when and where exposure may have taken place. The earliest human exposures to WNV identified in Manitoba were in mid-June 2006. While Canadian health officials are not predicting that an epidemic will occur this summer, they are finding more *Culex tarsalis* mosquitoes, which carry WNV, and the numbers are soaring early in the season. In June 2006, just two *C. tarsalis* mosquitoes were found in the Winnipeg area. This year the *C. tarsalis* number jumped to 389. The rising numbers prompted officials in Winnipeg to order fogging trucks to conduct mass sprayings of malathion to help control the insect population. In 2006, 151 human cases of West Nile virus were reported across Canada: 39 in Alberta; 19 in Saskatchewan; 50 in Manitoba; 42 in Ontario and one in Québec . (Source: Manitoba Office of the Chief Officer of Health, 6/21/07; CTV.ca, 7/2/07)

West Nile Virus was detected for the first time last month in sentinel chickens in Puerto Rico. The chickens were in four separate habitats in rural and urban areas. Seroconversion for WNV was detected by a chicken specific MAC-ELISA in seven sentinel chickens in four pens (one wetland, one mangrove forest, and two evergreen forests) from a sample drawn on June 4, 2007 and from another 21 chickens on June 11 in 11 out of the 12 pens, covering all types of habitats, including rural and urban areas. Puerto Rican health officials now are testing mosquitoes captured around the positive pen areas in an attempt to isolate the virus and the Puerto Rico Department of Health (PRHD) has alerted the population to take prevention measures. The Centers for Disease Control and Prevention and PRDH have conducted routine island-wide human surveillance for WNV since late 2002. To date, no human cases of WNV infection have been reported in Puerto Rico. More active surveillance now has been initiated. (Source: ProMed Digest, 7/3/07) ♦

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研究報告の概要

○韓国におけるマラリアPCRを用いたマラリア遡及プログラム

背景:マラリアは世界的に重大な感染症であり、主にハマダラカの刺咬により伝播するが、輸血によっても伝播する可能性がある。

目的:韓国はマラリア流行地域ではないが、近年一部の地域(特に韓国北部)で三日熱マラリアが多発している。我々は、供血後にマラリア感染の診断を受けた供血者の保管血液検体を調べ、輸血によるマラリア感染の発生について調査した。

方法:韓国疾病対策予防センター(KCDC)が、全国の病院や保健所からマラリア感染の報告を収集した。診断前6ヶ月間以内に供血を行ったマラリア患者の保管検体を選定しPCRを実施した。結果が陽性の場合、PCR陽性の血液成分を輸血された全受血者の血液検体を入手し、末梢血塗抹標本(PBS)、マラリア抗原・抗体検査、PCR及びnested PCRで検査した。

結果:2005年5月～2006年8月の期間に、合計2,056名の患者に三日熱マラリアの感染が確認された。このうち、46名(2.2%)に診断前直近6ヶ月以内の供血歴があった。この46名の供血回数は51回であった。保管血液検体51のうち、マラリアPCR陽性となったのは4名の供血者由来の5検体であった。PCR陽性となった供血後マラリアと診断されるまでの期間は、3～149日(中央値48日)であった。この5つの供血血液由来の7製剤(pRBC5製剤、PC2製剤)が7名の受血者に輸血されていた。pRBC製剤は供血から7～30日後、PC製剤は供血から2日後に輸血されていた。受血者7名のうち、1名はnested PCRでマラリア感染が認められた。残り6名の検査結果はいずれも陰性であった。

結論:マラリア遡及プログラムにより、1名の受血者に輸血によるマラリア伝播が確認された。PCRに基づくマラリア遡及調査は、輸血によるマラリア伝播の特定に役立つ。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
韓国のマラリア遡及プログラムにより、2005年5月～2006年8月の期間で1名の受血者に輸血によるマラリア伝播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問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、韓国のうちソウルより北の地域、江原道、京畿道をマラリア流行地(B地域)として、旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

Poster Session
6.3 Blood asafety - transfusion transmitted disease (TTD) - parasites

P239

MALARIA LOOKBACK PROGRAM USING MALARIA-PCR IN KOREA

Kim TY

Center for disease control and prevention, Seoul, South-Korea

Background: Malaria is a major communicable disease worldwide and mainly transmitted by the bite of infected Anopheles mosquitoes. But, malaria can be transmitted by blood transfusion.

Aims: Korea is not endemic area of malaria. Recently, Plasmodium vivax infection frequently occurred in some area, especially a north part of Korea. We investigated the stored blood samples of blood donors who were diagnosed as malaria infection after blood donations to evaluate the occurrence of transfusion-transmitted malaria infection.

Method: Korea center for disease control and prevention (KCDC) collected the reports of malaria infection from the hospitals or public health care centers nationwide. The stored blood samples of malaria patients who had donated blood within 6 months before diagnosis of malaria were selected. KCDC performed malaria-PCR for above selected blood samples. If the results of PCR for stored blood samples were positive, we obtained the blood samples of all recipients who had received PCR-positive blood components and performed peripheral blood smear(PBS) including thin and thick smear, malaria antigen and antibody, PCR and nested PCR for detection of malaria infection.

Results: Between May 2005 and Aug 2006, a total 2056 patients were confirmed to be Plasmodium vivax infection. Among 2056 patients with malaria, 46 tients (2.2%) had the history of blood donations within 6 months before the diagnosis of malaria. The number of blood donation for 46 patients was 51. Among 51 stored blood samples, five stored blood samples, which were originated from four donors had positive result of malaria PCR. The duration from first donation of PCR-positive blood to the diagnosis of malaria was 3–149 days (median 48 days). Seven blood components [five of packed red blood cell (pRBC), two of platelet concentration (PC)] were derived from five donated blood and given to seven recipients. Among PCR-positive blood components, PCR-positive pRBC were given to the recipients 7–30 days after blood donation and PC were given 2 days after blood donation. Among seven recipients who received PCR-positive blood components, only one recipient was proven to become infected with malaria using nested-PCR of malaria. The results of PBS, malaria antibody and antigen, PCR, and nested-PCR for other six recipients were all negative.

Conclusion: One recipient was documented as transfusion-transmitted malaria infection by malaria lookback program. Malaria lookback program-based on malaria PCR is useful to identify transfusion-transmitted malaria infection.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 8. 23	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Nomura T, Fujimoto T, Ebisutani C, Horiguchi H, Ando S. Jpn J Infect Dis. 2007 Jul;60(4):241-3.	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○淡路島の日本紅斑熱死亡例について

日本紅斑熱は、1984年に初めて報告されて以来、わが国におけるリケッチア感染症の中で重要な位置を占めている。淡路島南部の諭鶴羽山系は、*Rickettsia japonica*の好発地域の一つである。急速に進行し、DIC、消化管出血により死亡した日本紅斑熱症を報告する。

77歳男性、2005年9月2日より食欲低下、翌日より下腿に皮疹が出現、5日に38.7℃の高熱、歩行障害、構音障害が出現し、7日に症状が悪化したために受診した。自宅の畑には出ていたが、特に山林には出入りしていなかった。両下腿の皮膚に7mm大紅斑が散在、右肩前面にダニ刺し口があった。入院時検査で血小板減少、軽度の肝障害と脱水を認め、CRP20.34mg/dlと著明な上昇を認めた。リケッチア感染症による肝機能障害、DIC を疑い、ミノサイクリン200mg／日、ヘパリン1万単位／日、また脱水に対し補液を開始した。第2病日9月8日にはCRP17.7mg/dlとなり、炎症所見と肝酵素も改善傾向にあったが、9日急速に血圧低下、赤色凝血塊混じりの下血を繰り返し、全身皮膚にも紫斑が出現、その後心停止、死亡した。病理解剖の結果、両側胸水と胃～大腸粘膜からのoozing様出血を認めた。初診時のEDTA採血よりDNAを抽出し、PCR、nested-PCRを実施したところ、塩基配列は*R. japonica*と100%一致した。血清中の*R. japonica* IgGおよびIgM抗体価は、間接蛍光抗体法でIgG 320倍、IgM 80倍であった。

発疹性の発熱疾患であるリケッチア感染症は、わが国ではつつが虫病と日本紅斑熱が知られている。臨床症状からの鑑別は困難だが、発生時期や発生場所により疫学的な鑑別がある程度可能である。この症例では刺し口、発疹、発熱と3主徴がそろい、発症時期、発症地より日本紅斑熱を疑い、速やかにミノサイクリン投与を開始し、一時的に改善を認めたもののDICによる出血傾向により不幸な転帰をとった。

日本紅斑熱患者の2/3は50歳以上で比較的高齢者に多い疾患である。これは疾患感受性によるものというより、山村部の高齢化という環境要因が強いと考えられる。近年日本紅斑熱は増加傾向にあり、中山間地域の高齢化のため、今後も日本紅斑熱による重症例、死亡例は増加することが考えられる。医療従事者への啓発、検査の普及、同時に一般住民に対する注意勧告が必要と考える。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
淡路島で、急速に進行し、DIC、消化管出血により死亡した日本紅斑熱の症例報告である。	今後も情報の収集に努める。

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Laboratory and Epidemiology Communications

The First Fatal Case of Japanese Spotted Fever Confirmed by Serological and Microbiological Tests in Awaji Island, Japan

Tetsuhiko Nomura, Tsuguto Fujimoto[1]*, Chikara Ebisutani, Hidehisa Horiguchi and Shuji Ando[1]

Hyogo Prefectural Awaji Hospital, Hyogo 656-0013, and
[1]National Institute of Infectious Diseases, Tokyo 162-8640, Japan

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Japanese spotted fever is an important rickettsial disease in Japan (1). Japanese spotted fever was first reported by Mahara et al. (2,3) in 1984 in Anan City, Tokushima Prefecture, Japan. Since then, cases of Japanese spotted fever have been reported in many regions of the country. Yuzuruha Mountain in Awaji Island (Figure 1) is one of the areas heavily contaminated with *Rickettsia japonica*, and Japanese spotted fever cases are reported every summer in this area (4). In the present report, we describe the first fatal case of Japanese spotted fever confirmed by serological and microbiological methods.

A 77-year-old male recognized loss of appetite as the initial symptom on September 2, 2005, which is defined as day 1 of his illness. Rash appeared on the lower thighs on day 2, and a high fever of 38.7°C, dysarthria, and gait disorder on day 4. The patient visited Awaji hospital on day 6, because the symptoms had worsened. The patient claimed that he had worked on farmland, but had not visited a forested area before he developed the illness. He was alert on arrival at the

*Corresponding author: Mailing address: Infectious Disease Surveillance Center, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan. Tel: +81-3-5285-1111. Fax: +81-3-5285-1129, E-mail: fujimo-t@nih.go.jp

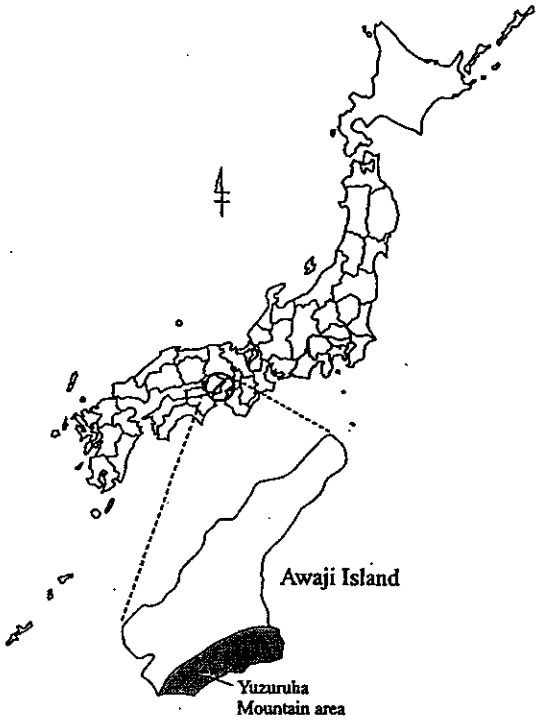

Fig. 1. Location of Yuzuruha Mountain area in Awaji Island.

hospital. At the first visit, other general findings included a height 160 cm, weight 50 kg, body temperature 36.4°C, blood pressure 102/58 mm Hg, pulse rate 86/min and regular, and SpO_2 97%. There were no abnormal findings in the chest region, and neither abdominal mass nor hepatosplenomegaly was palpable. Lymph node swelling was not found. No neurological abnormality was observed. Diffuse erythema of 7 mm in diameter was present on each bilateral lower thigh, and a bite mark (eschar) of a tick was evident on his anterior right shoulder.

Laboratory data were as follows: red blood cell count 4.48 $\times 10^6/\mu l$, Hb 13.8 g/dl, Ht 39.1%, white blood cell count 12,500/μl, platelet count 52,000/μl – thrombocytopenia observed, FDP 54 μg/ml, suggesting concurrent DIC. Mild hepatopathy and dehydration and a marked elevation of CRP at 20.34 mg/dl were observed. Weil-Felix reaction was negative: OX19<1:80, OX2<1:20, and OXK<1:20. Blood sugar level was elevated at 462 mg/dl and HbA1c 6.7%. As an underlying disease, concurrent diabetes was suspected based on the high blood sugar level. Findings from diagnostic imaging were noncontributory.

Because of the presence of an eschar and rash, it was suspected that liver dysfunction and DIC were due to rickettsial infection. Minocycline 200 mg/day, heparin 10,000 units/day, and fluid replacement for dehydration were started for treatment. On day 7, the 2nd day of hospitalization, CRP decreased to 17.7 mg/dl. Physical findings of inflammation started to improve and liver enzyme levels started to normalize. On day 8, blood pressure was 84/48 mm Hg, showing a rapid decline. Thereafter, the patient had repeated bloody stool mixed with red blood clots, and purpura appeared over the entire body. The patient eventually had cardiac arrest and death was confirmed on the same day. The autopsy demonstrated bilateral pleural effusion and oozing hemorrhage from the mucous membranes of the stomach to the large intestine.

DNA was extracted from the blood in EDTA collected at the initial examination. PCR (5) was performed with the R1-R2 primer combination to detect spotted fever group rickettsia, and a 2nd PCR was then performed using the Rj5-Rj10 primer combination, which specifically amplifies *R. japonica*. Agarose electrophoresis detected the target size of 357 bp as the amplified product. The nucleotide sequence of the amplified product was analyzed by direct sequencing. The nucleotide sequence matched 100% with that of *R. japonica* (GenBank accession no. U83442). Serum IgG and IgM antibody titers were examined against *R. japonica* by indirect immunofluorescence assay. The IgG and IgM titers were 1:320 and 1:80, respectively.

Tsutsugamushi disease and Japanese spotted fever are two diseases caused by rickettsial infection in Japan (1). Within Awaji Island, these diseases occur in different regions: tsutsugamushi disease in the northern part of the island and Japanese spotted fever in the southern Yuzuruha Mountain area (4). This is probably due to the different distribution of the respective vectors. There have been several cases of either disease annually. It is very difficult to differentiate these two diseases based on clinical symptoms, but they can be epidemiologically differentiated to some degree based on the time of year and the geographical location in which they occur. The patient in this study presented all 3 of the major signs for rickettsial infection, eschar, rash, and fever. The patient was clinically suspected to have Japanese spotted fever, according to the time and location of the incidence. Therefore, minocycline administration was started promptly, and the patient's conditions temporarily improved. However, the patient died from hemorrhaging manifestation.

The case fatality ratio of rickettsiosis by the spotted fever group is low. Rocky Mountain spotted fever has an exceptionally high case fatality rate of 3.7% (6). Kodama et al. (7) reported the first fatal case of Japanese spotted fever in 2001 in Awaji Island, but they did not confirm *Rickettsia* infection serologically. The patient in the present report was an elderly person. Although diabetes mellitus was not observed in his medical history, diabetes was thought to be the underlying disease according to the elevated blood sugar level and a high level of HbA1c. Therefore, it is likely that the patient was in an immunocompromised state, and this condition was one of the factors which contributed to the fatal outcome. For those who are old and predicted to have poor prognosis, steroid therapy has been reported to be effective, and the use of an antibacterial agent of the new quinolone group should be considered in combination with minocycline from the start of the treatment (1).

There is no gender difference in the reported cases of Japanese spotted fever. People of all age groups can be affected, but two-thirds of the patients are 50 years old or older. This is probably due to the strong social activity of the elderly people in the mountain village, rather than disease sensitivity. In recent years, the numbers of Japanese spotted fever cases have been increasing (8). Considering the increase in the percentage of the elderly population in semi-mountainous areas in Japan, it is likely that severe and fatal cases of Japanese spotted fever will increase in the future. On the other hand, healthcare providers are not provided enough information regarding this disease. Further, specific serodiagnostic tests and PCR are not widely used. Therefore, it is important to facilitate the use of these diagnostic techniques, and to provide the general residents with sufficient information concerning this disease.

This article appeared in the Infectious Agents Surveillance

Report (IASR), vol. 27, p. 36-37, 2006 in Japanese.

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医薬品 研究報告 調査報告書

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				2007. 8. 24	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	朝鮮日報. 2007 Aug 21.	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社)			韓国

研究報告の概要

○韓国でツツガムシ病患者急増

このところ、ツツガムシ病の患者が急増している。8月20日、疾病管理本部の発表によると、2002年に1,919人だったツツガムシ病の患者数が、04年は4,698人、06年には6,420人に増加したことが分かった。1993年末に法定伝染病に指定されて以来、患者数は実に25倍以上増加した。

ツツガムシ病は、主に9月以降、ツツガムシ菌に感染したツツガムシ(ダニの一種)の幼虫に刺されることにより感染する。10日間程度の潜伏期を経ると、突然高熱が発生し、目の充血、頭痛、筋肉痛、発疹などの症状が現れる。また、刺口がただれ、黒いかさぶたとなる場合もある。ツツガムシという名前は、ダニを意味する日本語(恙虫)に由来する。

サムスンソウル病院感染内科のペク・ギョンラン教授は、「抗生物質で比較的容易に治療できるが、初期症状が風邪と似ているため、適切な治療を受けずにそのまま放置すると、心不全や肺炎で死亡する危険性もある。秋にひどい風邪の症状が現れ、虫に刺された所があったり発疹が出たりした場合には、直ちに病院で治療を受けたほうがよい」と話した。

疾病管理本部伝染病監視チームのパク・ヘギョン研究員は、ツツガムシ病患者が増加したことに対し、「最近、伝染病管理が強化され、患者が確実に報告されるようになった上、温暖化によりダニの活動期間が長くなったためと思われる」と話した。ツツガムシ病は、農村に住む人たちが感染することが多かったが、最近では登山などアウトドア活動が活発になったことを受け、一般の患者も増えている。

ツツガムシ病を予防するには、ダニに刺されないよう長袖の服を着たり、靴下を履くなど皮膚の露出を最大限抑えなければならない。また秋に入り野外に出る際は、草地に直接座ったり、草むらで用を足したりすることは控えなければならない。

報告企業の意見	今後の対応
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使用上の注意記載状況・その他参考事項等

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ツツガムシ病は、主に9月以降、ツツガムシ菌に感染したツツガムシ(ダニの一種)の幼虫に刺されることにより感染する。10日間程度の潜伏期を経ると、突然高熱が発生し、目の充血、頭痛、筋肉痛、発疹などの症状が現れる。また、刺口がただれ、黒いかさぶたとなる場合もある。ツツガムシという名前は、ダニを意味する日本語(恙虫)に由来する。

サムスンソウル病院感染内科の白敬薗(ペク・ギョンラン)教授は、「抗生物質で比較的容易に治療できるが、初期症状が風邪と似ているため、適切な治療を受けずにそのまま放置すると、心不全や肺炎で死亡する危険性もある。秋にひどい風邪の症状が現れ、虫に刺された所があったり発疹が出たりした場合には、直ちに病院で治療を受けたほうがよい」と話した。

疾病管理本部伝染病監視チームのパク・ヘギョン研究員は、ツツガムシ病患者が増加したことに対し、「最近、伝染病管理が強化され、患者が確実に報告されるようになった上、温暖化によりダニの活動期間が長くなったためと思われる」と話した。ツツガムシ病は、主に野山で畑仕事をする農夫や、農村に住む人たちが感染することが多かったが、最近では登山などアウトドア活動が活発になったことを受け、一般の患者も増えている。

ツツガムシ病を予防するには、ダニに刺されないよう長袖の服を着たり、靴下を履くなど皮膚の露出を最大限抑えなければならない。また秋に入り野外に出る際は、草地に直接座ったり、草むらで用を足したりすることは控えなければならない。

・智[illegible]・ジヘ)記者

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研究報告の概要

本総説ではヒトプリオン病に関する最新の知見として，弧発性，家族性あるいは変異型クロイツフェルト・ヤコブ病（vCJD）（ウシの BSE を発症させるものと同じプリオン株によってヒトに発症する）のように後天性であるかどうかについて要約している。これらの複合的な神経変性障害の根底にある分子メカニズムは，宿主にコードされたプリオン蛋白（PrP^{c}）が異常アイソフォーム（PrP^{Sc}）へと変換するためであると考えられている。PrP^{Sc} は正常プリオン蛋白と比較すると，蛋白分解酵素感受性及び界面活性剤可溶性の変化を示す。PrP^{Sc} は伝播性プリオンの主要な，且つ，恐らく唯一の成分（蛋白単独モデル）であると考えられている。ヒト PrP 129 番目の遺伝子多型（メチオニン又はバリンをコードしている）はプリオン病の感受性に大きく影響を及ぼし，メチオニンホモ接合体は弧発性及び後天性クロイツフェルト・ヤコブ病（CJD）に罹患しやすいことがわかっている。興味深いことに，ヘテロ接合体の個体は，パプアニューギニアにおけるクールー病の最高齢の生存者であることが確認されている〔クールー病は食人の慣習（カニバリズム）により伝播された後天性プリオン病である〕。さらに，これまでに調査した vCJD の臨床症例はいずれもメチオニン 129 ホモ接合体であった。PrP の 129 残基は，PrP^{Sc} 又はその前駆体の立体構造，あるいはその形成における動態に影響を及ぼす可能性が高い。

弧発性 CJD とは対照的に，vCJD は若年患者に発現し，罹患期間が長い。双方とも同様の神経病理学的特性（海綿状変性，神経細胞の脱落及び星状細胞増加症）が認められるが，ほとんどの PrP アミロイド斑は vCJD で認められる。ヒトプリオン病でみられる臨床病理学的な不均質性は，様々な物理化学的特性を持つ独特の PrP^{Sc} アイソフォームに変換させる特異的プリオン株によって生じると考えられる。本総説には，現在までに確認された 4 種の主要な PrP^{Sc} の型が記載されている。1～3 型は典型的な CJD で認められる一方，4 型は唯一 vCJD で認められ，異なるグリコシル化プロファイルならびに蛋白分解酵素消化後の特異的なパターンを示す。さらに，PrP^{Sc} の異常アイソフォームの伝播は宿主ゲノムによって影響を受けていると考えられ，プリオン接種源もまた疾患の表現型に影響を及ぼしている可能性がある。家族性及び弧発性 CJD とは対照的に，vCJD ではリンパ細網組織内で PrP^{Sc} が容易に検出される。vCJD の脳で認められる 4 型の PrP^{Sc} とは異なる，特異的な型である 4t 型は vCJD 患者の扁桃腺で確認されている。リンパ細網組織には神経侵襲前に感染するため，扁桃生検により明確かつ早期の vCJD 診断が可能である。

依然として不明な点が多く，クールー病が 40～50 年にわたる長い潜伏期を伴うことを考慮すると，ヒト vCJD の伝播性の程度を確実に予測することはできない。そのため，vCJD プリオンの二次的（ヒト間の）伝播を回避するための最大限の注意が必要である。

使用上の注意記載状況・その他参考事項等

BYL-2007-0283

報告企業の意見	今後の対応
この総説は，ヒトプリオン病の知見とその複雑な疾患の重要点を要約している。現時点で，多くの不明点が残っているが，ヒト間での vCJD 感染を予防する為には，あらゆる予防策を講じることが賢明であると考える。	プリオン病の伝播リスクについて，引き続き情報収集に努める。

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Review

Update on human prion disease

Jonathan D.F. Wadsworth, John Collinge *

MRC Prion Unit and Department of Neurodegenerative Disease, Institute of Neurology, University College London, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK

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Abstract

The recognition that variant Creutzfeldt–Jakob disease (vCJD) is caused by the same prion strain as bovine spongiform encephalopathy in cattle has dramatically highlighted the need for a precise understanding of the molecular biology of human prion diseases. Detailed clinical, pathological and molecular data from a large number of human prion disease patients indicate that phenotypic diversity in human prion disease relates in part to the propagation of disease-related PrP isoforms with distinct physicochemical properties. Incubation periods of prion infection in humans can exceed 50 years and therefore it will be some years before the extent of any human vCJD epidemic can be predicted with confidence.
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Keywords: Bovine spongiform encephalopathy; Creutzfeldt-Jakob disease; Fatal familial insomnia; Gerstmann-Sträussler-Scheinker disease; Kuru; Prion; Prion disease; Prion protein; Transmissible spongiform encephalopathy; Variant Creutzfeldt-Jakob disease

1. Introduction

Prion diseases are fatal neurodegenerative disorders that include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker disease (GSS), fatal familial insomnia (FFI), kuru and variant CJD (vCJD) in humans [1,2]. Their central feature is the post-translational conversion of host-encoded, cellular prion protein (PrP^C), to an abnormal isoform, designated PrP^{Sc} [1,2]. This transition appears to involve only conformational change rather than covalent modification and confers PrP^{Sc} with partial resistance to proteolytic degradation and detergent insolubility. Human prion diseases are biologically unique in that the disease process can be triggered through inherited germline mutations in the human prion protein gene (*PRNP*), infection (by inoculation, or in some cases by dietary exposure) with prion-infected tissue or by rare sporadic events that generate PrP^{Sc} [2–4]. Substantial evidence indicates that an abnormal PrP isoform is the principal, if not the sole, component of the transmissible infectious agent, or prion [1,2,5,6]. The mechanism of neurodegeneration that accompanies the accumulation of PrP^{Sc} in the brain remains unknown [7] although there is increasing evidence that this may involve an apoptotic mechanism involving inhibition of the proteosome [8]. The existence of multiple strains or isolates of prions has been difficult to accommodate within a protein-only model of prion propagation and understanding how a protein-only infectious agent can encode distinct disease phenotypes in humans has been of considerable biological interest. A wealth of experimental evidence now suggests that prion strain diversity is encoded within PrP itself and phenotypic diversity in human prion diseases relates to differing physicochemical properties of abnormal PrP isoforms [9–18].

2. Aetiology and clinical features of human prion diseases

Human prion diseases can be divided aetiologically into inherited, sporadic and acquired forms [1,2]. Human prion diseases with distinct aetiologies are associated with a range of clinical presentations which are now seen as clinico-pathological syndromes rather than individual disease entities [3,4,19].

3. Sporadic CJD

Approximately 85% of cases of human prion disease occur sporadically as Creutzfeldt–Jakob disease (sporadic CJD) at a

* Corresponding author. Tel.: +44 20 7837 4888; fax: +44 20 7837 8047.
E-mail address: j.collinge@prion.ucl.ac.uk (J. Collinge).

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rate of 1–2 cases per million population per year across the world, with an equal incidence in men and women [20,21]. The aetiology of sporadic CJD is unknown, although hypotheses include somatic *PRNP* mutation [20,22,23], or the spontaneous conversion of PrP^{C} into PrP^{Sc} as a rare stochastic event [22]. Polymorphism at residue 129 of human PrP (encoding either methionine (M) or valine (V)) powerfully affects susceptibility to human prion diseases [21,24–28]. About 38% of Europeans are homozygous for the more frequent methionine allele, 51% are heterozygous, and 11% homozygous for valine. Homozygosity at *PRNP* codon 129 predisposes to the development of sporadic and acquired CJD [21,24–28]. Most sporadic CJD occurs in individuals homozygous for this polymorphism. This susceptibility factor is also relevant in the acquired forms of CJD, most strikingly in vCJD; all clinical cases studied so far have been homozygous for codon 129 methionine of *PRNP* (see below). Additionally, a *PRNP* susceptibility haplotype has been identified indicating additional genetic susceptibility to sporadic CJD at or near to the *PRNP* locus [29,30].

Classical sporadic CJD presents as a rapidly progressive multifocal dementia usually with myoclonus. The onset is usually in the 45–75 years age group with median age at death of 68 years [21]. The clinical progression is typically over weeks progressing to akinetic mutism with a median disease duration of 5 months [21]. Prodromal features, present in around a third of cases, include fatigue, insomnia, depression, weight loss, headaches, general malaise and ill-defined pain sensations. In addition to mental deterioration and myoclonus, frequent additional neurological features include extrapyramidal signs, cerebellar ataxia, pyramidal signs and cortical blindness. Raised cerebrospinal fluid 14-3-3 protein, neuronal specific enolase (NSE), and S-100, although not specific for CJD, may be helpful diagnostically in the appropriate clinical context [21,31–36]. The electroencephalogram (EEG) may show characteristic pseudoperiodic sharp wave activity that is helpful in diagnosis but present only in around 60% of cases [21]. In contrast cortical signal changes on diffusion weighted MRI are extremely helpful in the diagnosis of CJD [37–42]. Neuropathological confirmation of CJD is by demonstration of spongiform change, neuronal loss and astrocytosis. PrP amyloid plaques are usually not present in CJD [43] although PrP immunohistochemistry, is nearly always positive [43–45].

Atypical forms of sporadic CJD are well recognised. 10% of cases of CJD have a much more prolonged clinical course with a disease duration of over 2 years [46]. Around 10% of CJD cases present with cerebellar ataxia rather than cognitive impairment, so-called ataxic CJD [47]. Heidenhain's variant of CJD refers to cases in which cortical blindness predominates with severe involvement of the occipital lobes. The panencephalopathic type of CJD refers to cases with extensive degeneration of the cerebral white matter in addition to spongiform vacuolation of the gray matter and has been predominately reported from Japan [47–49]. Amyotrophic variants of CJD have been described with prominent early muscle wasting. However, most cases of dementia with amyotrophy are not experimentally transmissible and their relationship with CJD is unclear [50,51].

4. Inherited prion disease

Approximately 15% of human prion diseases are associated with autosomal dominant pathogenic mutations in *PRNP* [14,22,52–54]. How pathogenic mutations in *PRNP* cause prion disease has yet to be resolved, however, in most cases the mutation is thought to lead to an increased tendency of PrP^{C} to form PrP^{Sc}, although there is evidence to suggest that this may not be solely attributable to decreased thermodynamic stability of mutated PrP^{C} [55,56]. Experimentally manipulated mutations of the prion gene can lead to spontaneous neurodegeneration without the formation of detectable protease resistant PrP [57,58]. These findings raise the question of whether all inherited forms of human prion disease invoke disease through the same mechanism, and in this regard it is currently unknown whether all are transmissible by inoculation [52].

Over 30 autosomal dominant pathogenic *PRNP* mutations have been described [2,3,53,54]. In the appropriate clinical setting identification of a pathogenic *PRNP* mutation provides diagnosis of inherited prion disease and sub-classification according to mutation; *PRNP* analysis is also used for pre-symptomatic genetic testing in affected families [4,52,54,59]. Traditionally, inherited prion diseases have been classified by the presenting clinical syndrome, falling into three main subdivisions of either GSS, CJD or FFI. GSS classically presents as a chronic cerebellar ataxia with pyramidal features with dementia occurring much later in a clinical course that is typically longer than that seen in classical CJD [52]. Fatal familial insomnia (FFI) is characterised by progressive untreatable insomnia, dysautonmoia and dementia, selective thalamic degeneration and is most commonly associated with a missense mutation at codon 178 of *PRNP* [60], although sporadic FFI with no causative mutation in *PRNP* has been reported [61,62]. Remarkably, some families show extensive phenotypic variability which can encompass both CJD- and GSS-like cases as well as other cases which do not conform to either CJD or GSS phenotypes [19,53,54,63–66]. Progressive dementia, cerebellar ataxia, pyramidal signs, chorea, myoclonus, extrapyramidal features, pseudobulbar signs, seizures and amyotrophic features can be seen in variable combinations. Such atypical prion diseases may lack the classical histological features of a spongiform encephalopathy entirely although PrP immunohistochemistry is usually positive [64]. The existence of phenotypic overlap between individuals with different mutations and even in family members with the same *PRNP* mutation indicates that accurate classification of inherited human prion diseases should be based upon mutation alone [19,67]. Because of the extensive phenotypic variability seen in inherited prion disease and its ability to mimic other neurodegenerative conditions, notably Alzheimer's disease, *PRNP* analysis should be considered in all patients with undiagnosed dementing and ataxic disorders [19,63,64,68].

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5. Acquired prion disease

Although the human prion diseases are transmissible diseases, acquired forms have, until recently, been confined to rare and unusual situations.

5.1. Iatrogenic CJD

The two most frequent causes of iatrogenic CJD occurring through medical procedures have arisen as a result of implantation of dura mater grafts and treatment with growth hormone derived from the pituitary glands of human cadavers [69,70]. Less frequent incidences of human prion disease have resulted from iatrogenic transmission of CJD during corneal transplantation, contaminated electroencephalographic (EEG) electrode implantation and surgical operations using contaminated instruments or apparatus [69,70]. The clinical presentation in iatrogenic forms of human prion disease appear to be related to their aetiology and in particular the route of exposure to human prions [52,70,71]. Peripheral routes of infection are typically associated with longer incubation periods and usually present with a kuru-like syndrome in which ataxia rather than dementia is the prominent early clinical feature. In contrast, patients with dura mater graft-related exposure to human prions, in which infectivity is placed in closed proximity to the brain, typically have a clinical presentation similar to that of sporadic CJD [72], although exceptions with unusual clinical features have been reported [73].

5.2. Kuru

The most well-known example of acquired prion disease in humans is kuru, transmitted by cannibalism among the Fore linguistic group of the Eastern Highlands in Papua New Guinea [28,52,74,75]. The central clinical feature of kuru is progressive cerebellar ataxia and in sharp contrast to sporadic CJD, dementia is late and may be absent. A prodrome and three clinical stages consisting of an ambulatory stage, a sedentary stage and a tertiary stage have been described [52,74,75]. Remarkably, kuru demonstrates that incubation periods of infection with human prions can exceed 50 years [75]. *PRNP* codon 129 genotype has shown a pronounced effect on kuru incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes [27,28,75]. The clear survival advantage for codon 129 heterozygotes provides a powerful basis for selection pressure in the Fore [28,75]. However, an analysis of worldwide haplotype diversity and allele frequency of coding and non-coding polymorphisms of *PRNP* suggests that balancing selection at this locus (in which there is more variation than expected because of heterozygote advantage) is much older and more geographically widespread [28]. Evidence for balancing selection has been shown in only a few human genes. With biochemical and physical evidence of cannibalism on five continents, one explanation is that cannibalism resulted in prion disease epidemics in human prehistory, thus imposing balancing selection on *PRNP* [28].

5.3. Variant CJD

The appearance of a novel human prion disease, variant CJD (vCJD), in the United Kingdom from 1995 onwards [76], and the experimental confirmation that this is caused by the same prion strain as that causing BSE in cattle [10,77–79], has led to widespread concern that exposure to the epidemic of BSE poses a distinct and conceivably a severe threat to public health in the United Kingdom and other countries [4,80]. The extremely prolonged and variable incubation periods seen with prion diseases when crossing a species barrier means that it will be some years before the parameters of any human epidemic can be predicted with confidence [75,80–83]. In the meantime, we are faced with the possibility that significant numbers in the population may be incubating this disease and that they might pass it on to others via blood transfusion, blood products, tissue and organ transplantation and other iatrogenic routes [80,84–87].

To date, the clinical presentation of human BSE infection has only been recognised as vCJD in *PRNP* codon 129 methionine homozygotes. The neuropathological features of vCJD are somewhat different from those seen in classical (sporadic or iatrogenic) CJD, and are characterised by the presence of abundant florid PrP plaques [76,88] and the propagation of type 4 PrP^{Sc} in brain [10,15] (Fig. 1). The early clinical presentation of vCJD resembles kuru more than classical CJD and consists of behavioral and psychiatric disturbances, peripheral sensory disturbance and cerebellar ataxia. Common early psychiatric features include depression, withdrawal, anxiety, insomnia, and apathy. Neurological symptoms have preceded psychiatric symptoms in a minority of cases. No common early neurological features have been reported, but paraesthesiae and/or pain in the limbs is seen in around half of the cases. However a significant proportion of patients exhibited neurological symptoms within 4 months of clinical onset, and these included poor memory, pain, sensory symptoms, unsteadiness of gait and dysarthia. Disorientation, hallucinations, paranoid ideation, confabulation, impaired self-care, and the commonest neurological features (cerebellar signs, chorea, dystonia, myoclonus, upper motor neuron signs and visual symptoms), develop late in the course of the illness [89]. The duration of disease is longer in vCJD with mean patient survival times of about 14 months [36], compared with about 5 months for classical CJD [21]. Moreover, whereas classical CJD is predominantly a late onset disease with a median age at death of 68 years [21], the median age of onset of vCJD is 26 years [36,89]. The EEG is not helpful in the diagnosis of vCJD, whilst generalised slowing is usually present, the characteristic periodic changes associated with classical CJD are not. The CSF 14-3-3 protein is not helpful, and may often be negative [90]. The most useful non-invasive investigation in advanced cases of vCJD has been MR neuroimaging, particularly the FLAIR sequence [91]. Early case reports noted bilateral increased signal in the posterior thalamus (pulvinar) on T2 weighted images [92]. A retrospective review of MR scans from 36 histologically confirmed cases of vCJD using mainly other forms of human prion disease subjects as controls suggested

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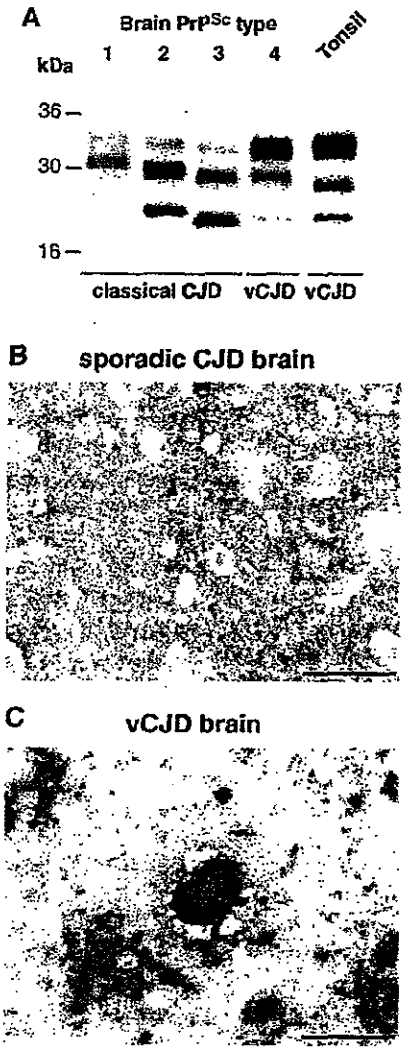

Fig. 1. Characterisation of disease related prion protein in human prion disease. (A) Immunoblots of proteinase K digested tissue homogenate with anti-PrP monoclonal antibody 3F4 showing PrP^{Sc} types 1–4 in human brain and PrP^{Sc} type 4t in vCJD tonsil. Types 1–3 PrP^{Sc} are seen in the brain of classical forms of CJD (either sporadic or iatrogenic CJD), while type 4 PrP^{Sc} and type 4t PrP^{Sc} are uniquely seen in vCJD brain or tonsil, respectively. (B, C) Brain from patients with sporadic CJD or vCJD show abnormal PrP immunoreactivity following immunohistochemistry using anti-PrP monoclonal antibody ICSM35. Abnormal PrP deposition in sporadic CJD brain most commonly presents as diffuse, synaptic staining, whereas vCJD brain is distinguished by the presence of florid PrP plaques consisting of a round amyloid core of PrP surrounded by a ring of spongiform vacuoles. Scale bars B and C, 50 μm.

that the "pulvinar sign" occurred frequently in advanced cases of vCJD [93]. However, histologically confirmed cases of vCJD with minimal or absent pulvinar changes on MR neuroimaging at a mean 10.5 months during an illness of mean 15 months duration were identified in this series. The absence of characteristic MR findings does therefore not exclude a diagnosis of vCJD and more recently figures of 81% sensitivity and 94% specificity have been reported in a series of patients including 27 cases of vCJD diagnosed by tonsil biopsy [94]. As these studies suggest, the pulvinar sign is not specific for vCJD and these MRI appearances are described in sporadic CJD [95], paraneoplastic limbic encephalitis [96] and in a number of rare conditions [97–100]. In contrast, a firm tissue based diagnosis of vCJD can be made during life by tonsil biopsy, with demonstration of a characteristic sub-type of PrP^{Sc} (see below).

6. Molecular basis of phenotypic variability in human prion disease

The marked clinical heterogeneity observed in human prion diseases has yet to be explained. However, it has been clear for many years that distinct isolates, or strains, of prions can be propagated in the same host and these are biologically recognised by distinctive clinical and pathological features [2,101,102]. It is therefore likely that a proportion of clinicopathological heterogeneity seen in sporadic CJD and other human prion diseases relates to the propagation of distinct human prion strains. Within the framework of the protein-only hypothesis of prion propagation, distinct clinical and neuropathological phenotypes are thought to be determined by the propagation of distinct PrP^{Sc} isoforms with divergent physicochemical properties [1,2,9–11,103,104]. Furthermore the propagation of distinct abnormal PrP isoforms may be determined by the host genome [79,105,106].

To date we have identified four major types of human PrP^{Sc} associated with sporadic and acquired human prion diseases that can be differentiated on immunoblots after limited proteinase K digestion of brain homogenates [10,12,15] (Fig. 1). PrP^{Sc} types 1–3 are seen in classical (sporadic or iatrogenic) CJD brain, while type 4 PrP^{Sc} is uniquely seen in vCJD brain [10,12,15]. An earlier classification of PrP^{Sc} types seen in classical CJD described only two banding patterns [11] with PrP^{Sc} types 1 and 2 that we describe corresponding with the type 1 pattern of Gambetti and colleagues, and our type 3 fragment size corresponding to their type 2 pattern [13,107]. While type 4 PrP^{Sc} is readily distinguished from the PrP^{Sc} types seen in classical CJD by a predominance of the di-glycosylated PrP glycoform, type 4 PrP^{Sc} also has a distinct proteolytic fragment size [15] although this is not recognised by the alternative classification which designates type 4 PrP^{Sc} as type 2b [107].

In addition to the identification of distinct human PrP^{Sc} types associated with sporadic and acquired prion disease, molecular strain typing has provided insights into the phenotypic heterogeneity seen in inherited human prion diseases [53,108]. In agreement with existing evidence that human prion strain diversity is generated through variance in PrP^{Sc} conformation and glycosylation, cases of inherited prion disease caused by point mutations have glycoform ratios of PrP^{Sc} fragments distinct from those seen in both classical CJD [108–112] and vCJD [108]. Individuals with the same *PRNP* mutation can also propagate PrP^{Sc} with distinct fragment sizes [108,109,113]. Notably however the detection of PrP^{Sc} in the molecular mass range of ca. 21–30 kDa is by no means a consistent feature. Instead, some cases, in particular those in which amyloid plaques are a prominent feature, show smaller protease resistant fragments of ca. 7–15 kDa [53,108–110,113,114]. The

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propagation of pathological isoforms of wild type PrP may also make a significant contribution to phenotypic variability in inherited prion disease [23,115–117]. These collective data indicate that PrP^{Sc} isoforms may be generated in inherited prion disease with unique physicochemical properties, reflected by sensitivity to proteinase K digestion or PrP^{Sc}/prion infectivity ratios that are very different from the PrP^{Sc} types propagated in sporadic and acquired forms of human prion disease. For example in P102L inherited prion disease, it is now apparent that three isoforms of protease resistant PrP with divergent physicochemical properties can be propagated. Two distinct abnormal conformers derived from PrP 102L generate protease-resistant fragments of either ca. 21–30 kDa or 8 kDa [23,108–110,118], while abnormal conformers of wild type PrP appear to generate proteolytic fragments of only ca. 21–30 kDa [23]. Glycoform ratios of ca. 21–30 kDa proteolytic fragments generated from PrP 102L and wild type PrP are not only distinct from each other, but are also distinct from those generated from wild type PrP in sporadic or acquired CJD [23]. Differences in neuropathological targeting of these distinct disease-related PrP species, together with differences in their abundance and potential neurotoxicity, provides a molecular mechanism for generation of multiple phenotypes in P102L inherited prion disease. Clearly, new experimental methods may be required in order to fully document the spectrum of abnormal PrP isoforms seen in inherited prion disease. In this context it is important to note that not all inherited prion disease may invoke disease through the same mechanism. For example the *PRNP* A117V mutation gives rise to transmembrane forms of PrP (PrP^{ctm}) which it is proposed may invoke a neurological disease without generation of PrP^{Sc} [58]. It is also not presently known whether all inherited prion diseases are transmissible by inoculation.

Efforts to produce an unified international classification and nomenclature of human PrP^{Sc} types has been complicated by the fact that the N-terminal conformation of some PrP^{Sc} subtypes seen in sporadic CJD can be altered *in vitro* via changes in metal-ion occupancy [12,15] or solvent pH [119–121]. Additionally there is now increasing evidence for co-existence of different PrP^{Sc} types within the same brain [13,15,21,23,108–110,122–127]. While it has recently been proposed that pH alone determines the N-terminal structure of PrP^{Sc} in sporadic CJD [120,121], this interpretation has not been supported by other studies [12,17,124], and specific PrP^{Sc} conformations show critical dependence upon the presence of copper or zinc ions under conditions where pH 7.4 is strictly controlled [12]. Although agreement has yet to be reached on methodological differences, nomenclature and the biological importance of relatively subtle biochemical differences in PrP^{Sc}, there is strong agreement between laboratories that phenotypic diversity in human prion disease relates in significant part to the propagation of disease-related PrP isoforms with distinct physicochemical properties [9–18,124,127,128]. Importantly, it is now also becoming apparent that protease-sensitive pathological isoforms of PrP may have a significant role in both animal and human prion disease [18,104,129,130] and therefore development of new diagnostic tests that do not rely on protease digestion are required. In this context, the conformation-dependent immunoassay [104] has recently been reported to have high diagnostic sensitivity in human prion disease [18].

The hypothesis that alternative conformations or assembly states of PrP provide the molecular substrate for clinicopathological heterogeneity seen in human prion diseases (and that this relates to the existence of distinct human prion strains) has been strongly supported by transmission experiments to conventional and transgenic mice. Transgenic mice expressing only human PrP with either valine (V) or methionine (M) at residue 129 have shown that this polymorphism constrains both the propagation of distinct human PrP^{Sc} conformers and the occurrence of associated patterns of neuropathology [10,77,79,131,132]. Biophysical measurements suggest that this powerful effect of residue 129 on prion strain selection is likely to be mediated via its effect on the conformation of PrP^{Sc} or its precursors or on the kinetics of their formation, as it has no measurable effect on the folding, dynamics or stability of PrP^{C} [133]. These data are consistent with a conformational selection model of prion transmission barriers [2,80,131,134] (a model also recently supported by work with yeast prions [135]) and strongly support the "protein only" hypothesis of infectivity by suggesting that prion strain variation is encoded by a combination of PrP conformation and glycosylation [2]. These findings also provide a molecular basis for *PRNP* codon 129 as a major locus influencing both prion disease susceptibility and phenotype in humans.

Notably, our transgenic modeling studies indicate that there is no common preferred PrP^{Sc} conformation for V129 and M129 human PrP that can be generated as a result of exposure to the vCJD/BSE prion strain [131]. Depending on the origin of the inoculum and the PrP codon 129 genotype of the host, primary or secondary transmission of BSE-derived prions can result in four distinct prion disease phenotypes. Transgenic mice homozygous for human PrP M129 propagate either type 2 or 4 PrP^{Sc} with respective neuropathologies consistent with human sporadic CJD or vCJD [79,131], whereas transgenic mice homozygous for human PrP V129 either propagate type 5 PrP^{Sc} and a distinct pattern of neuropathology, or develop clinical prion disease in the absence of detectable PrP^{Sc} [77,131]. Depending on the source of the inoculum, multiple disease phenotypes are also seen in human PrP codon 129MV heterozygous transgenic mice [132]. However in the PrP codon 129MV genotype, none of these phenotypes resemble the neuropathological phenotype of vCJD as the propagation of type 4 PrP^{Sc} is dissociated from the occurrence of abundant florid PrP plaques [132]. While caution must be exercised in extrapolating from animal models, even where faithful recapitulation of molecular and pathological phenotypes is possible [79,131] (Fig. 2), our data, together with closely similar findings of Manson and colleagues [136], argue that primary human BSE prion infection, and secondary infection with vCJD prions by iatrogenic routes, may not be restricted to a single disease phenotype. Stratification of all human prion disease cases by PrP^{Sc} type will enable rapid recognition of any change in relative frequencies of particular PrP^{Sc} sub-types in

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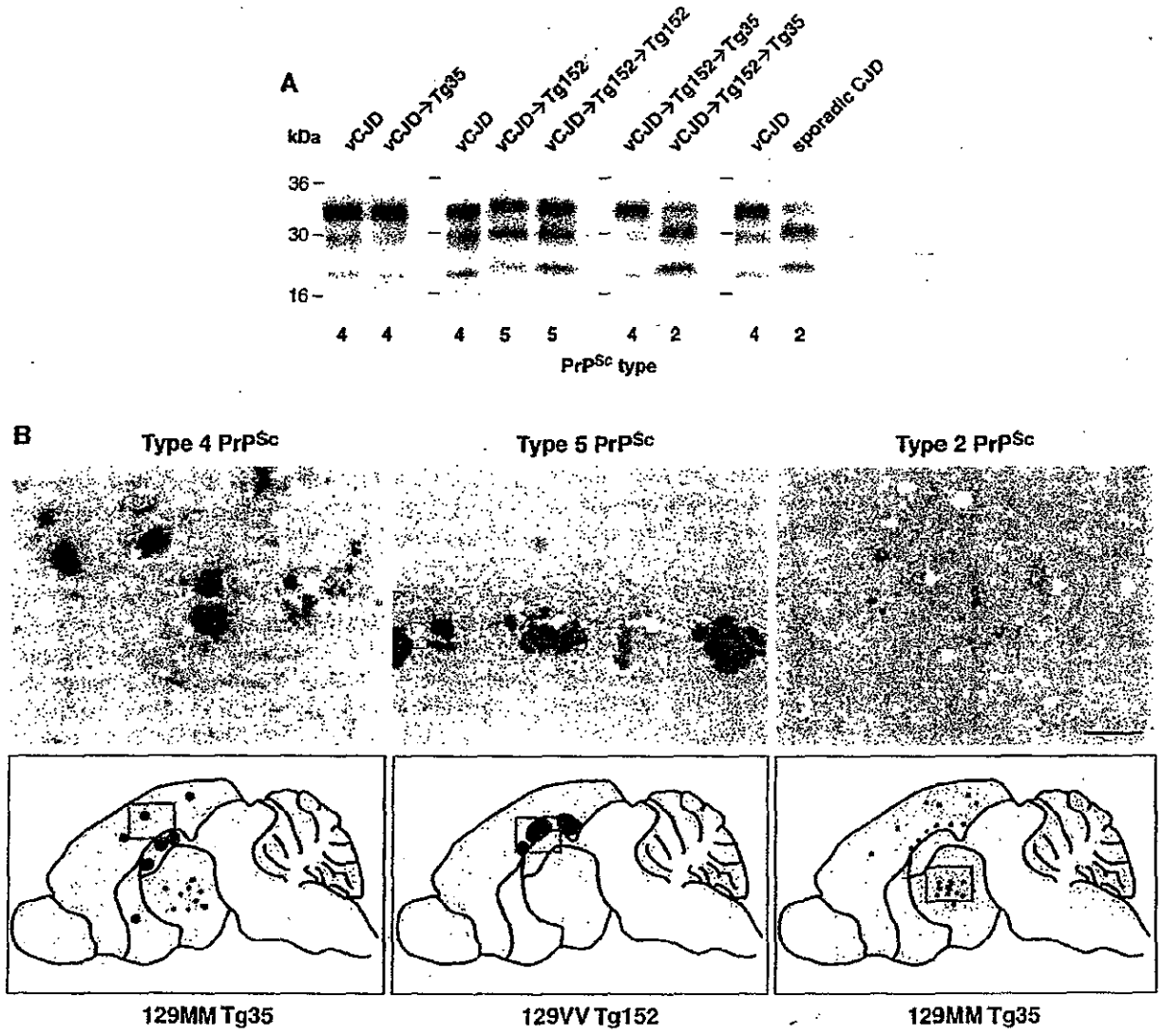

Fig. 2. Recapitulation of vCJD molecular and neuropathological phenotype in transgenic mice. Primary transmission of vCJD prions to transgenic Tg (HuPrP129M$^{+/+}$*Prnp*$^{o/o}$)-35 mice (Tg35) results in faithful propagation of type 4 PrP^{Sc} and the occurrence of abundant florid PrP plaques that are the neuropathological hallmark of vCJD. In contrast, primary transmission of vCJD prions to transgenic Tg(HuPrP129V$^{+/+}$*Prnp*$^{o/o}$)-152 mice (Tg152) produces type 5 PrP^{Sc} and a distinct pattern of neuropathology that is maintained after secondary passage in Tg152 mice but after passage in Tg35 mice induces propagation of either type 4 or type 2 PrP^{Sc} and respective neuropathologies consistent with vCJD or sporadic CJD. (A) Representative immuno-blots of proteinase-K treated brain homogenates from variant and sporadic CJD (*PRNP* 129 MM genotype) and transgenic mice analysed with anti-PrP monoclonal antibody 3F4. The identity of the brain sample is designated above each lane with the type of PrP^{Sc} present in the sample designated below. (B) Representative immunohistochemical analysis of transgenic mouse brain showing abnormal PrP immunoreactivity, including PrP-positive plaques, stained with anti-PrP monoclonal antibody 3F4. Scale bar: 100 μm. Lower panels show the regional distribution of abnormal PrP deposition. Green boxes in the sketches denote the area from which PrP stained sections are derived.

relation to either BSE exposure patterns or iatrogenic sources of vCJD prions [2,79,131,132].

7. Peripheral pathogenesis in human prion disease

Concomitant with studies on PrP^{Sc} from brain has been investigation of PrP^{Sc} in peripheral tissues in human prion diseases. These studies have established that the pathogenesis of vCJD differs significantly from that of other forms of CJD. PrP^{Sc} is readily detectable in lymphoreticular tissues in vCJD and not in classical CJD or inherited prion disease [84,108,137–142]. The fact that tonsillar prion infection has not been detected in iatrogenic CJD associated with use of human cadaveric derived pituitary hormone [138] or kuru (our unpublished data) argues that the distinctive pathogenesis of vCJD relates to the effect of prion strain rather than to a peripheral route of infection.

Depending upon the density of lymphoid follicles, PrP^{Sc} concentrations in vCJD peripheral tissues can vary enormously, with levels relative to brain as high as 10% in tonsil [84,138] or as low as 0.002% in rectum [84]. A distinctive PrP^{Sc} type, designated type 4t, is seen in both ante-mortem and post-

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mortem tonsil from patients with vCJD [84,138] (Fig. 1) including secondary vCJD infection resulting from blood transfusion [87]. Type 4t PrP^{Sc} in tonsil differs in the proportions of the PrP glycoforms from type 4 PrP^{Sc} seen in vCJD brain [84,138] implying the superimposition of tissue and strain specific effects on PrP glycosylation [80,138]. As infection of lymphoreticular tissues is thought to precede neuroinvasion, and indeed has been detected in archived surgical samples removed prior to development of vCJD [81,143], tonsil biopsy is likely to allow firm diagnosis at the early clinical stage or indeed pre-clinically [4]. Early diagnosis obviates the need for further investigation, which may include brain biopsy, necessary to exclude alternative potentially treatable conditions and will be increasingly important with the advent of putative treatments and clinical trials where the aim must be to intervene early before extensive CNS damage has occurred [4]. To date tonsil biopsy has shown has shown 100% sensitivity and specificity for diagnosis of clinical vCJD [84,94,137,138] (and our unpublished data).

The distinctive peripheral pathogenesis of vCJD has provided the basis for prevalence screening of the general population for vCJD infection. Two anonymous screens of lymphoreticular tissues, removed during routine surgery, have been performed [81,82]. Tonsil appears a more sensitive reporter of vCJD prion infection than appendix [144] and it is of concern that three positives appendix specimens were reported from a retrospective screen of around 12 000 largely appendix samples [81]. A pilot scale prospective study of 2000 tonsils found no positives, despite use of high sensitivity methods [82]. However, this negative result cannot provide reassurance that significant community infection is unlikely because of the relatively small sample size, demographic and age-related factors and unknown test sensitivity during the prolonged incubation period [82]. Accordingly, a national large scale study is now being organized by the UK Department of Health which aims to screen 100,000 tonsils for disease-associated PrP to estimate prevalence of vCJD prion infection in the UK population.

8. Secondary transmission of vCJD prions

The unknown but potentially high prevalence of clinically silent infection with BSE prions allied with the distinctive pathogenesis of vCJD has led to significant concerns that there may be substantial risks of iatrogenic transmission of vCJD prions via blood products and contaminated surgical and medical instruments [80,84,142,145].

Indeed three cases of transfusion-associated vCJD prion infection have already been reported from amongst a very small cohort of known exposed individuals [85–87]. Prions resist many conventional sterilisation procedures and surgical stainless steel-bound prions transmit disease with remarkable efficiency when implanted into mice [146,147].

Precautionary risk reduction measures have already been taken with respect to blood and blood products [85,148] however major concerns remain relating to possible iatrogenic transmission of vCJD prions via contaminated surgical instruments. Transmission of vCJD to conventional mice involves a transmission barrier that severely limits the ability to detect low titre infectivity [77–79,149]. Pending development of a transmission barrier-free model for bioassay of vCJD prions, high sensitivity immunodetection of PrP^{Sc} has been used to provide an upper limit on PrP^{Sc} levels in peripheral tissues, including blood, to inform risk assessment models [84,141,142]. High concentrations of PrP^{Sc} appear to be largely confined to the central nervous system and lymphoreticular tissues in vCJD. While a range of surgically important tissues have levels of PrP^{Sc} 10^4–10^5 fold lower than found in brain [84], the demonstration of prion infectivity in vCJD rectum despite barely detectable levels of PrP^{Sc} is consistent with PrP^{Sc}/prion infectivity ratios in vCJD being closely similar to experimental rodent prion strains [145]. These findings strongly endorse the value of PrP^{Sc} analysis in vCJD for defining risk reduction strategies to limit secondary transmission of vCJD prions and reinforce concerns that iatrogenic transmission of vCJD prions might occur through contaminated endoscopes, biopsy forceps or surgical instruments [142,145,147,150,151].

Predictions of the eventual size of a vCJD epidemic have varied widely, although some recent estimates, based on current cases of vCJD, suggest that the total epidemic may be relatively small [152]. However, key uncertainties, notably with respect to major genetic effects on the incubation period [54,105], suggest the need for caution. Importantly, such models cannot estimate the number of infected individuals, which remains unknown. The mean incubation period for kuru has been estimated to be around 12 years [75] with a similar estimate in iatrogenic CJD associated with the use of human-cadaver-derived pituitary growth hormone [70]. However, the maximum incubation periods in kuru can exceed 50 years [75]. The transmission barrier of BSE between cattle and human beings is unknown and cannot be directly measured. However, the cattle-to-mouse barrier for BSE has been well characterised experimentally by comparative endpoint titration. BSE prions transmit readily to laboratory mice, including after oral dosing [153]. The murine LD_{50} (lethal dose causing 50% mortality) in C57Bl/6 mice is about 500-fold higher than that in cattle [154]; this barrier also results in a three-fold to four-fold increase in mean incubation period. Mean incubation periods of human BSE infection of 30 years or more should therefore be regarded as possible, if not probable, with the longest incubation periods approaching (and perhaps exceeding) the typical human lifespan [75]. The mean incubation periods of secondary vCJD (involving human-to-human transmission) would however be expected to have considerably shorter mean incubation periods than in primary vCJD resulting from exposure to BSE prions. The incubation periods seen in the first clinical cases of secondary vCJD are 6 and 6.5 years [85,87] suggesting the shortest incubation periods of primary vCJD will be much longer, particularly as the oral route of transmission is also typically associated with longer incubation periods than parenteral routes. A preliminary estimate of the shortest incubation periods of primary vCJD, based on the youngest cases seen, is around 12 years which would be consistent with such extrapolation from the shortest incubation periods in secondary vCJD [75].

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It remains unclear if individuals with the other *PRNP* codon 129 genotypes, VV and MV, will also succumb to clinical disease with longer mean incubation periods after primary infection with BSE prions or secondary transmission of vCJD prions. The *PRNP* 129MV blood transfusion recipient had evidence of prion infection of lymphoreticular tissues at autopsy but died from a ruptured abdominal aortic aneurysm with no reported evidence of a neurological disorder; the brain showed no pathological features of vCJD [86]. It is unclear if this individual would have gone on to develop clinical prion disease had they lived longer and if so would have had the phenotype of vCJD. Subclinical or carrier states of prion infection are recognised in animal models [134,155], including BSE or vCJD prion-inoculated transgenic mice expressing human prion proteins [79,131,132,136]. Modelling susceptibility in such "humanised" transgenic mice suggests such genotypes will be susceptible but may develop disease phenotypes distinct from vCJD because of selection and propagation of different prion strain types [131,132]. The absence of detectable brain PrP^{Sc} in the 129 MV blood transfusion patient precluded molecular strain typing of prions. Although splenic PrP^{Sc} from this case resembled that seen in vCJD, this required use of phosphotungstic acid precipitation of PrP^{Sc} which interferes with molecular strain typing [84]. In addition, type 5 PrP^{Sc}, seen in vCJD-inoculated transgenic mice expressing human PrP 129V, but not yet documented in humans, has a similar glycoform profile [77,131,132]. It is possible that this patient was propagating a prion strain distinct from that causing vCJD. Importantly, Peden et al. noted that they were unable to detect PrP^{Sc} in lymphoid follicles in tonsil, appendix or large intestine from this patient, although abnormal PrP was detected in spleen and in a cervical lymph node. As tonsillar involvement has to date been invariably seen in clinically affected cases of vCJD, Peden et al. speculated that this case may represent a distinctive pathogenesis related to route of exposure (intravenous rather than the oral route of exposure to BSE prions presumed in primary vCJD). It is also possible that the absence of a species barrier in secondary (human to human) infection may affect phenotype. However the detection of type 4t PrP^{Sc} in the tonsil of the most recent codon 129MM transfusion case [87] argues against these interpretations, and suggests that absence of tonsillar PrP^{Sc} in the MV blood recipient may be due to the effect of *PRNP* genotype and perhaps selection of a distinctive prion strain. These findings highlight the importance of the need for continued surveillance and investigation of all forms of human prion disease within the UK and other populations with extensive dietary exposure to BSE prions.

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研究報告の概要

本研究で我々は、異なるポアサイズのウイルス除去膜を使用し、異なる処理を行ったスクレーピープリオン蛋白（PrP^{Sc}）の除去能力を評価した。たん白質を含まない系で超音波処理を行わなかった 263K 由来のミクロソーム画分（263K MF）をろ過すると、75nm 以下のポアサイズのウイルス除去膜ろ過により PrP^{Sc} がウエスタンブロット（WB）の検出限界以下まで除去された。しかし、超音波処理により粒子径分布を至適化するように調製した 263K MF をスパイク物質として使用したときは、75nm のろ液中に PrP^{Sc} が検出された。
血漿由来調製品存在下で超音波処理により粒子径分布を至適化するように調製した 263K MF をろ過すると、15nm のろ過のみが全ての条件で WB の検出限界以下まで PrP^{Sc} が除去されることが示された。しかし、1 条件下の 15nm ろ液のバイオアッセイの結果では、感染性 PrP^{Sc} が確認された。
この結果は、スパイク物質の性質が、プリオン除去のためのフィルター能力の評価において重要な要素であること、および超音波処理又は界面活性剤処理のような、スパイクするプリオン粒子サイズの分布が最小になるように設計された方法をスパイク物質の調製に使用しなければならないことを示している。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 略
1) 略
2) 略
3) 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
プリオンソースの処理の違いにより、ウイルス除去膜による PrP^{Sc} の除去効果が異なるとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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Prion removal by nanofiltration under different experimental conditions

Mikihiro Yunoki [a,b,*], Hiroyuki Tanaka [b], Takeru Urayama [a,b], Shinji Hattori [b], Masahiro Ohtani [b], Yuji Ohkubo [b], Yoshiyasu Kawabata [b], Yuuki Miyatake [b], Ayako Nanjo [b], Eiji Iwao [c], Masanori Morita [b], Elaine Wilson [d], Christine MacLean [d], Kazuyoshi Ikuta [a]

[a] *Department of Virology, Research Institute for Microbial Diseases, Osaka University, Japan*
[b] *Research & Development Division, Benesis Corporation, Japan*
[c] *Pharmaceutical Research Division, Mitsubishi Pharma Corporation, Japan*
[d] *BioReliance, Invitrogen BioServices, UK*

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Abstract

Manufacturing processes used in the production of biopharmaceutical or biological products should be evaluated for their ability to remove potential contaminants, including TSE agents. In the present study, we have evaluated scrapie prion protein (PrP^{Sc}) removal in the presence of different starting materials, using virus removal filters of different pore sizes. Following 75 nm filtration, PrP^{Sc} was detected in the filtrate by Western blot (WB) analysis when a "super-sonicated" microsomal fraction derived from hamster adapted scrapie strain 263K (263K MF) was used as the spike material. In contrast, no PrP^{Sc} was detected when an untreated 263K MF was used. By using spike materials prepared in a manner designed to optimize the particle size distribution within the preparation, only 15 nm filtration was shown to remove PrP^{Sc} to below the limits of detection of the WB assays used under all the experimental conditions. However, infectious PrP^{Sc} was recovered following 15 nm filtration under one experimental condition. The results obtained suggest that the nature of the spike preparation is an important factor in evaluating the ability of filters to remove prions, and that procedures designed to minimize the particle size distribution of the prion spike, such as the "super-sonication" or detergent treatments described herein, should be used for the preparation of the spike materials.
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Keywords: Prion; Removal; Filter; Clearance study; Spike material

1. Introduction

The transmission of variant Creutzfeldt–Jakob disease (vCJD) through blood transfusion has been of increasing concern, since a fourth possible transmission case was reported [1]. In addition, prions have been detected in the buffy coat separated from the blood of hamsters infected with scrapie, using a biochemical assay (protein misfolding cyclic amplification, or PMCA) [2]. Infectious prions are thought to be the causative agent of the transmissible spongiform encephalopathy (TSE) diseases, which include Creutzfeldt–Jakob disease (CJD), vCJD, and bovine spongiform encephalopathy (BSE). Therefore, to reduce the risk of transmission when raw materials for protein products (such as plasma) are contaminated with infectious prions, measures should be introduced to decrease the prion load, to evaluate the risk to the product, and to introduce prion removal/inactivation step(s) in the manufacturing process, if feasible [3–5]. Unlike viruses, the minimum infectious prion unit does not exist as a single particle. The infectious prion unit is believed to be composed of protein polymers/aggregates, rather than a prion particle. The unusual nature of the prion agent makes it particularly important to

* Corresponding author. Hirakata Research Laboratory, Research & Development Division, Benesis Corporation, 2-25-1 Shodai-Ohtani, Hirakata, Osaka 573-1153, Japan. Tel.: +81 72 850 0100; fax: +81 72 864 2341.
E-mail address: yunoki.mikihiro@mk.m-pharma.co.jp (M. Yunoki).

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consider the effect of the prion spike material when evaluating process steps for prion clearance. A rationale for the choice of the spike preparation used for such evaluation studies should be provided [4].

Several prion strains have been used to evaluate manufacturing processes for their ability to remove TSE agents, including hamster scrapie prion protein (PrP^{Sc}, 263K or Sc237), and mouse PrP^{BSE} (301V). In a polyethylene glycol (PEG) fractionation process, hamster PrP^{Sc} and human PrP^{vCJD}, prepared using the same methodology, were reported to behave in a very similar manner [6]. Different prion spike preparations have been used to investigate prion removal, including crude brain homogenate (BH), microsomal fraction (MF), caveolae-like domains (CLDs), and purified PrP^{Sc}. Of these materials, purified PrP^{Sc} was reported to behave differently from the other preparations in an 8% ethanol fractionation step [7]. This result suggests that the methods used to prepare the prion spike material may be a critical factor in prion clearance studies. Furthermore, these reports are useful in providing a rationale for the choice of the prion source and spike preparation used for such evaluation studies [8].

Tateishi et al. reported that sarkosyl influenced the ability of BMM40 filters to remove prions, using BH derived from CJD-infected mice [9]. The presence of sarkosyl was also shown to significantly reduce the capacity of Planova (P)-35N to remove the scrapie agent ME7, while filtration with P-15N resulted in the complete removal of infectivity, to below the limit of detection of the bioassay used, in both the presence and absence of sarkosyl [10]. Van Holten et al. evaluated the capacity of Viresolve 180 membranes (designed for virus removal from proteins of <180 kDa) to remove prions by using BH which was lysolecithin-treated, sonicated, and subsequently passed through a 100 nm filter (SBH), and demonstrated removal of PrP^{Sc} down to the limit of detection of the Western blot assay used. They argued that by using a better defined spike material, where the size of the scrapie particles was limited, the results may be more relevant with respect to the removal of potential TSE infectivity in plasma than previous studies that used a less well-defined BH [11].

Aggregation of the prion protein is a critical parameter when evaluating nanofiltration steps. The actual form of the infectious agent present in plasma in natural infection is not known. In addition, nanofiltration is typically performed late in the downstream processing, after protein purification steps, which may result in removal of larger or aggregated prion forms. Therefore, use of a spike preparation containing large aggregates may result in an over-estimate of the prion removal capacity of a filter. Although the reports described above, and others, have shown excellent prion removal ability for a number of filters, most reports have not described the particle size distribution of the prion protein in the spike preparations used. Therefore, in this study we have investigated the prion removal capacity of P-35N, P-20N and P-15N filters under diverse conditions, considering the particle size distribution of the MF preparations used.

2. Materials and methods

2.1. Preparation of microsomal fraction (MF)

Brains removed from hamsters infected with scrapie strain 263K [12] (originally obtained from the Institute for Animal Health, Edinburgh, UK), were homogenized in phosphate buffered saline (PBS) until homogeneous, to a final concentration of 10% (w/v). The homogenate was clarified by low speed centrifugation, to remove larger cell debris and nuclei, and the supernatant material was then further clarified by centrifugation at 8,000 × *g* for 10 min at 4 °C, before being ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, to concentrate the scrapie fibrils, and small membrane vesicles and fragments. The pelleted material was resuspended in PBS, aliquoted, and stored at −80 °C. This material was designated 263K MF. Prior to use, stocks were thawed at 37 °C, and sonicated 2 × 4 min on ice water (Ultrawave ultrasonic bath model #U100, 130 W 30 kHz, Ultrawave Ltd., Cardiff, UK). Six independent batches of 263K MF were used in this study. These batches are designated 263K MF preparation lots A–F (Tables 1–3). Normal MF, derived from normal (i.e. uninfected) hamster brain material, was also prepared as described above.

Since we were unable to measure the particle size distribution of contaminated materials in our facility, we used normal MF, and investigated changes in the particle size distribution following strong sonication or treatment with detergent. Various concentrations of sarkosyl (*N*-lauroylsarcosine sodium salt, Nacalai Tesque, Inc., Kyoto, Japan), lysolecithin (L-α-lysophosphatidylcholine, Sigma-Aldrich Corp., St. Louis, USA), Triton X-100 (polyethylene glycol mono-*p*-isooctylphenyl ether, Nacalai Tesque, Inc.), TNBP (tri-n-butyl phosphate, Wako Pure Chemical Industries, Ltd., Osaka, Japan), and/or 1% Tween 80 (Nacalai Tesque, Inc.) were added to normal MF. Changes in the particle size distribution were then monitored by dynamic light scattering method using volume-weighted gaussian analysis using a submicrometer particle sizer (NICOMP Type 370, Particle Sizing Systems, Inc., Santa Barbara, USA). To evaluate the effect of strong sonication, normal MF was sonicated using a closed system ultrasonic cell disruptor (Bioruptor UCD-200T, CosmoBio Co. Ltd., Tokyo, Japan) with a resonance chip set in the tube. Sonication was performed for 1 min at 20 kHz, 200 W in a cold water-bath. Ten cycles of sonication were performed, with a 1 min

Table 1
Scrapie infectivity in different 263K MF preparations[a]

	Log_{10} LD_{50}/ml	SE at 95% probability
Non-super-sonicated 263K MF lot C	5.7	0.44
Super-sonicated 263K MF lot C	6.0	0.53
Super-sonicated 263K MF lot D	5.3	0.69
SD-treated, ultracentrifuged, super-sonicated and 220 nm-filtered 263K MF lot C	6.9	0.69

[a] This bioassay study was performed in accordance with GLP regulations.

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Table 2
Removal of PrP^{Sc} from PrP^{Sc}-inoculated PBS

	PVDF filter				Planova filter					
	220 nm		100 nm		P-75N (72 ± 2 nm)		P-35N (35 ± 2 nm)		P-15N (15 ± 2 nm)	
Super-sonicated	+	−	+	−	+	−	+	−	+	−
Before filtration	4.2/3.5[a]	3.5/4.2	4.2/3.5	3.5/4.2	4.2/4.2	3.5/4.2	4.2/4.2	3.5/4.2	4.2/4.2	3.5/4.2
Filtered	3.8/3.8	3.1/3.8	3.8/3.1	2.4/3.1	2.4/2.4	<1.0/<1.0	<1.0/<1.0	<1.0/<1.0	<1.0/<1.0	<1.0/<1.0
LRF[b]	0.4/−0.3	0.4/0.4	0.4/0.4	1.1/1.1	1.8/1.8	≥2.5/≥3.2	≥3.2/≥3.2	≥2.5/≥3.2	≥3.2/≥3.2	≥2.5/≥3.2

Data represents total PrP^{Sc} present in samples, expressed as $\log_{10}$ arbitrary units, following Western blot analysis as described for WB1. This study was performed in accordance with GLP regulations.

[a] Two independent batches of 263K MF were used: lot C (left) and lot D (right), respectively.

[b] LRF, log reduction factor = total PrP^{Sc} in input/total PrP^{Sc} in filtrate, expressed as a $\log_{10}$ value.

interval between each sonication treatment. During the treatment cycle, the particle size distribution was monitored. We named this treatment cycle "super-sonication".

Different preparations of 263K MF, treated with various combinations of detergent, ultracentrifugation and/or "super-sonication", were used as the spiking agent in the process evaluation studies, and are described in the relevant methods sections below.

2.2. Detection of PrP^{Sc} by Western blotting (WB)

To determine the relative levels of PrP^{Sc} present in different samples, WB assays were performed. Three slightly different WB methodologies were applied over the course of the studies, all of which are based on detection of the disease-associated, protease-resistant form of the prion protein (PrP^{Sc}), using the monoclonal antibody 3F4 (Signet Laboratories, Inc., Dedham, USA) [13]. WB methods 1 and 2 were developed independently, and use different approaches to calculate the titer of PrP^{Sc}. As these assays were performed as part of GLP studies intended for regulatory submission, the results are presented as reported in these studies.

2.2.1. Method 1 (WB1)

Samples and controls were either tested directly, or first ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, and the pelleted material then resuspended in PBS. Ultracentrifugation was performed to concentrate the PrP^{Sc} present in large volume samples, and to remove soluble proteins or buffer components that might interfere with the WB assay. Samples were digested with proteinase K (Roche Diagnostics, GmbH, Penzberg, Germany) for 60 min at 37 °C. The optimal concentration of proteinase K, to remove any background that could interfere with the detection of PrP^{Sc} and to allow effective recovery of the PrP^{Sc} protein, was previously established for each sample. Digested samples were mixed 1:1 with Laemmli sample buffer (62.5 mM Tris–HCl, pH 6.8, 25% (v/v) glycerol, 2% (w/v) SDS, and 0.01% (w/v) bromophenol blue, BioRad Laboratories Inc., Hercules, USA) containing 5% (v/v) β-mercaptoethanol. After boiling, serial 5-fold dilutions of

Table 3
Removal of PrP^{Sc} from PrP^{Sc}-inoculated plasma preparations[a]

Filter	P-35N (35 ± 2 nm)		P-20N (19 ± 2 nm)		P-15N (15 ± 2 nm)			
Preparation	IVIG	Haptoglobin	IVIG	Haptoglobin	Antithrombin			Thrombin
Spike material	263K sMF[c]	263K sMF[c]	263K sMF[d]	263K dsMF[e]	263K dMF[f]	263K sMF[c]		263K dsMF[e]
MF preparation lot.	C/D	B	E/F	E/F	A/A	B		C/D
Spike ratio	1/100	1/200	1/20	1/200	1/50	1/21		1/20
Detection method[b]	WB1	WB3	WB2	WB2	WB1	WB3	BA	WB1
Before filtration	3.2/2.5	2.4	6.8/6.8	6.7/6.1	3.1/3.1	3.6	+ve	3.7/3.7
Filtered	0.8/0.8	<1.0	4.8/4.3	4.8/4.7	0.0/0.0	<0.8	+ve	<0.2/<0.2
Log reduction factor	2.4/1.7	≥1.4	2.0/2.5	1.9/1.4	≥3.1/≥3.1	≥2.8	NA	≥3.5/≥3.5

Abbreviations used: 263K MF, microsomal fraction derived from hamster adapted scrapie strain 263K; IVIG, intravenous immunoglobulin; 263K sMF, "super-sonicated" 263K MF; WB, Western blotting; 263K dsMF, detergent treated and "super-sonicated" 263K MF; 263K dMF, detergent treated 263K MF; BA, bioassay; +ve, scrapie positive.

[a] Scaled down conditions were designed according to current guidelines. However, in a study using P-35N filter and haptoglobin, clogging of the filter occurred, and the filtration was subsequently terminated.

[b] WB1, WB2, and WB3 mean Western blotting methods 1, 2 and 3, respectively. The studies involving the use of WB1 and WB2 were performed in accordance with GLP regulations; the studies involving the use of WB3 and the qualitative BA shown in this table, were performed as non-GLP studies.

[c] 263K MF was "super-sonicated" then 220 nm-filtered prior to spiking.

[d] 263K MF was ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended in buffer equivalent to the starting material without protein, "super-sonicated", and 220 nm-filtered prior to spiking.

[e] 263K MF was "SD-treated", ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended in the starting material (thrombin) or saline (haptoglobin), and "super-sonicated". These materials were 220 nm-filtered prior to spiking.

[f] 263K MF was treated with 0.1% sarkosyl for 30 min at room temperature.

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the sample were then prepared and subjected to electrophoresis using 12% (w/v) SDS-polyacrylamide gels. Proteins were transferred from the gels to 0.45 μm PVDF membranes (Immobilon-P, Millipore Corp., Billerica, USA), and non-specific binding sites on the membranes were then blocked by overnight incubation in buffer containing dried milk and Tween 20. The blocked membranes were incubated with monoclonal antibody 3F4, washed extensively, and then incubated with a secondary horseradish peroxidase (HRP)-conjugated anti-mouse antibody (Sigma-Aldrich Corp.). After further extensive washing, bound antibody was detected using an ECL-Plus detection system (GE Healthcare UK Ltd, Buckinghamshire, UK) and exposure to blue-light sensitive film.

The level of PrP^{Sc} present in each sample was calculated based on the end-point dilution after analysis by WB. The end-point dilution for each titration was taken as the first dilution at which the 28 kDa PrP^{Sc} protein could not be detected. The reciprocal of this dilution was then taken as the titer of the agent, and expressed in arbitrary units/ml.

2.2.2. Method 2 (WB2)

WB was performed essentially as described by Lee et al. [14]. Briefly, samples were digested with proteinase K at approximately 6 U/ml for 60 min at 37 °C and centrifuged at approximately 20,000 × *g* for 60 min at 4 °C. The pellet was then resuspended and denatured in a 1:1 mix of supernatant and sample buffer (62.5 mM Tris–HCl, pH 6.8, 10% (v/v) glycerol, 2% (w/v) SDS and 0.0025% (w/v) bromophenol blue, Invitrogen Corp. Carlsbad, USA), by heating at approximately 100 °C. Serial 3.2-fold (0.5 $\log_{10}$) dilutions of the sample were prepared, and loaded onto 12% (w/v) SDS-polyacrylamide gels. Following electrophoresis, proteins were transferred to nitrocellulose membranes (Invitrogen Corp.), and the membranes blocked using buffer containing dried milk and Tween 20 for 1–2 h at room temperature. The blocked membranes were then incubated with monoclonal antibody 3F4, washed extensively, and incubated with a secondary alkaline phosphatase (AP)-conjugated anti-mouse antibody (Cambridge Biosciences Ltd., Cambridge, UK). After further extensive washing, bound antibody was detected using a CDP Star/Nitroblock II detection system (Applied Biosciences, Bedford, USA) and exposure to blue-light sensitive film.

The titer of PrP^{Sc} present in each sample was calculated slightly differently from WB1 and WB3. The end-point dilution for each titration was taken as the last dilution at which the 28 kDa PrP^{Sc} protein could be detected. The reciprocal of this dilution was then taken as the amount of agent in the sample volume tested, and was adjusted for the volume tested and any concentration factors, to give a titer/ml for the original process sample.

2.2.3. Method 3 (WB3)

Samples were ultracentrifuged twice at 150,000 × *g* for 1 h. The samples in the precipitates were then resuspended in PBS at 1/1 or 1/10th volume of the original. Resuspended samples were treated with proteinase K at a final concentration of 10–100 μg/ml. After incubation at 37 °C for 60 min, samples were treated with 10 mM 4-(2-aminoethyl)-benzene sulfonyl fluoride hydrochloride (AEBSF) at room temperature for 10 min, then mixed with 5× SDS-polyacrylamide gel electrophoresis (PAGE) sample buffer (300 mM Tris–HCl, 12% (w/v) SDS, 25% (v/v) glycerol, and 0.025% (w/v) bromophenol blue, pH 6.8, with 25% (v/v) β-mercaptoethanol) and heated at 100 °C for 5 min. Samples were serially 5-fold diluted with 1× PAGE dilution buffer (60 mM Tris–HCl, 2.4% (w/v) SDS, 5% (v/v) glycerol, and 0.005% (w/v) bromophenol blue, pH 6.8). SDS-PAGE was performed at 30 mA per gel for approximately 42 min. The proteins in the gel were transferred to 0.45 μm PVDF membranes. After treating with blocking buffer (5.0% (w/v) skimmed milk in PBS, 0.05% (v/v) Tween 20), the membrane was incubated with monoclonal antibody 3F4 at 4 °C overnight, then incubated with HRP-conjugated sheep anti-mouse IgG (Sigma-Aldrich Corp.). Bound antibody was visualized by chemiluminescence (ECL-Plus) on X-ray film. The titer of PrP^{Sc} present in the samples was calculated as described for method 1 in Section 2.2.1.

2.3. Evaluation of PrP^{Sc} removal by filtration

A 10% (v/v) concentration of "super-sonicated" 263K MF was prepared in PBS, and 10 ml aliquots were then filtered through a 220 nm or a 100 nm 4 cm^2 PVDF filter (Millex-GV or -VV, Millipore Corp.). In addition, 25 ml aliquots of "super-sonicated" 263K MF in PBS were filtered through a 0.01 m^2 P-75N (72 ± 2 nm), P-35N (35 ± 2 nm), or P-15N (15 ± 2 nm) filter (Asahi Kasei Medical Co., Ltd. Tokyo, Japan). Two independent batches of 263K MF were used. WB1 analysis of samples before and after filtration was performed to determine the removal of PrP^{Sc} under the different conditions. Non-sonicated 263K MF (from the same batch of 263K MF) was also filtered as a control.

2.4. Hamster bioassay to determine the infectious titer of 263K scrapie stocks

Three- to four-week-old female specific pathogen-free (SPF) Syrian hamsters were used in these experiments. Serial 10-fold dilutions of each sample or positive control were prepared in PBS. Six hamsters per sample dilution were inoculated intra-cerebrally with 0.02 ml per animal. The inoculated animals were monitored daily for general health, and weekly for clinical evidence of scrapie. Animals were euthanized once advanced signs of scrapie were evident, or at the end of the assay period (200 days). The brain was removed from each hamster following euthanasia: one half was fixed for histopathology and the other half was stored frozen at −70 °C for further analysis if required. For histopathological analysis, sections taken at four standard coronal levels, to cover the nine areas of the brain which are recognized to be mostly infected by the scrapie agent, were stained with hematoxylin and eosin, and scored for the presence or absence of scrapie lesions [15]. Histopathological analysis was performed on samples from around the clinical end-point of the titration assays, to confirm the clinical results. Hamsters that died during the

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course of the study for reasons other than scrapie infection were not included in the final calculation of infectious titers. Infectious titers were expressed as a 50% lethal dose (LD_{50}) according to the method of Kärber [16].

Samples taken before and after filtration during the P-15N/antithrombin (AT; previously named antithrombin-III) study were tested for the presence of scrapie infectivity using a qualitative hamster bioassay. Syrian hamsters were inoculated with undiluted samples only, as described above, except that only three animals were used per sample.

2.5. Evaluation of PrP^{Sc} removal in the presence of plasma preparations

To investigate whether differences in how the scrapie spike material was prepared influenced our evaluation of prion removal, two different spiked preparations were compared using the manufacturing process for preparing AT (Neuart®, Benesis Corp., Osaka, Japan). Samples taken during the actual manufacturing process, immediately before the Planova step, were spiked with 263K MF treated with 0.1% (w/v) sarkosyl for 30 min at room temperature, or with 220 nm-filtered "super-sonicated" 263K MF. The spiked AT materials were then passed through a P-15N filter. The influence of different filtration conditions on the removal of PrP^{Sc} was compared for the same spike preparations, and for different spike preparations, using heat/PEG-treated intravenous immunoglobulin (IVIG) (Venoglobulin-IH, Benesis Corp.) and haptoglobin (Haptoglobin Injection-Yoshitomi, Benesis Corp.). Samples taken during the actual manufacturing process, immediately before the Planova step, were spiked with: 220 nm-filtered "super-sonicated" 263K MF (IVIG/P-35N and haptoglobin/P-35N); 263K MF ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended in buffer equivalent to the starting material without protein, "super-sonicated" and 220 nm-filtered (IVIG/P-20N); or 263K MF treated with 0.3% (v/v) TNBP/1% (v/v) Tween 80 for 6 h at 30 °C ("SD treatment"), ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended in saline, "super-sonicated", and 220 nm-filtered (haptoglobin/P-20N). The spiked material was then passed through either a P-35N filter or a P-20N filter (19 ± 2 nm). Although not part of the manufacturing process for haptoglobin, the SD treatment was included for the spiked preparation in an effort to reduce the clogging of the filter that occurs following the addition of a prion spike. Filtration processes for the thrombin preparation (Thrombin-Yoshitomi, Benesis Corp.) were also investigated. For thrombin, a sample taken during the actual manufacturing process immediately before the Planova step was spiked with 263K MF subjected to "SD treatment" followed by ultracentrifugation at 141,000 × *g* for 60 min at 4 °C, resuspended in the starting material, "super-sonicated" and 220 nm-filtered, and the spiked material then passed through a P-15N filter.

The experimental conditions used in the prion removal studies were designed to mimic the conditions used during the actual manufacturing process for the relevant product. For all processes, samples were analyzed by WB. The $\log_{10}$ reduction factor (LRF) for PrP^{Sc} was calculated for each filtration run, by comparing the total amount of PrP^{Sc} present in samples before and after filtration. All studies involving the use of WB1 and 2, and the quantitative bioassays, were performed in facilities in compliance with current GLP regulations. Studies involving the determination of average particle size in normal MF preparations, the use of WB3, and the qualitative bioassay, were performed as non-GLP studies.

3. Results

3.1. Influence of MF preparation method on particle size distribution

Ideally, to represent a "worst case" challenge for a filter, the smallest form of prion protein, or infectious agent, should be used. Studies to investigate the optimum method for preparing the prion spike material were therefore performed. In these studies, changes in the average particle size in normal MF were investigated, as 263K-infected brain material could not be handled within our facility. Although prion particles in MF derived from 263K-infected brain material were not investigated directly, we tried to optimize the design of our experiments by minimizing the size of particles in normal MF, as particle size may influence filtration performance (both with respect to filter blockage, and removal of PrP^{Sc}). The results are shown in Figs. 1 and 2.

Treatment of normal MF with sarkosyl or lysolecithin reduced the average size of particles to approximately 100 nm, when 0.1% or higher concentrations of the detergents were used. However, below that concentration, the particle size did not change significantly, with the exception of 0.01% lysolecithin which reduced the average particle size to approximately 300 nm (Fig. 1A,B). Treatment with Triton X-100 did not result in a significant change in particle size, even at 1% (Fig. 1C). Treatment with 0.3% TNBP or 1% Tween 80 alone was not able to reduce the particle size below 200 nm. However, when combined, one of the conditions generally used for viral inactivation ("SD treatment"), 0.3% TNBP and 1% Tween 80 reduced the average particle size to below 200 nm (Fig. 1D). These results suggest that the reduction in average particle size in normal MF depends on the choice of detergent(s), and the concentration and combination of detergent(s) used.

We also studied the effect of "super-sonication" on the particle size in normal MF. The results showed that "super-sonication" could reduce the average particle size to a very fine level in a short time, without the need to change the composition of the normal MF material (Fig. 2A). Since "super-sonication" is a temporary physical procedure, reversal of the particle size reduction may possibly occur. To exclude this possibility during the experiments, we conducted a stability study on the particle size in normal MF after "super-sonication". There was no significant change in the particle size up to 24 h after "super-sonication", with the size remaining at approximately 100 nm (Fig. 2B).

The results showed that the particle size of normal MF preparations could be reduced significantly by treatment

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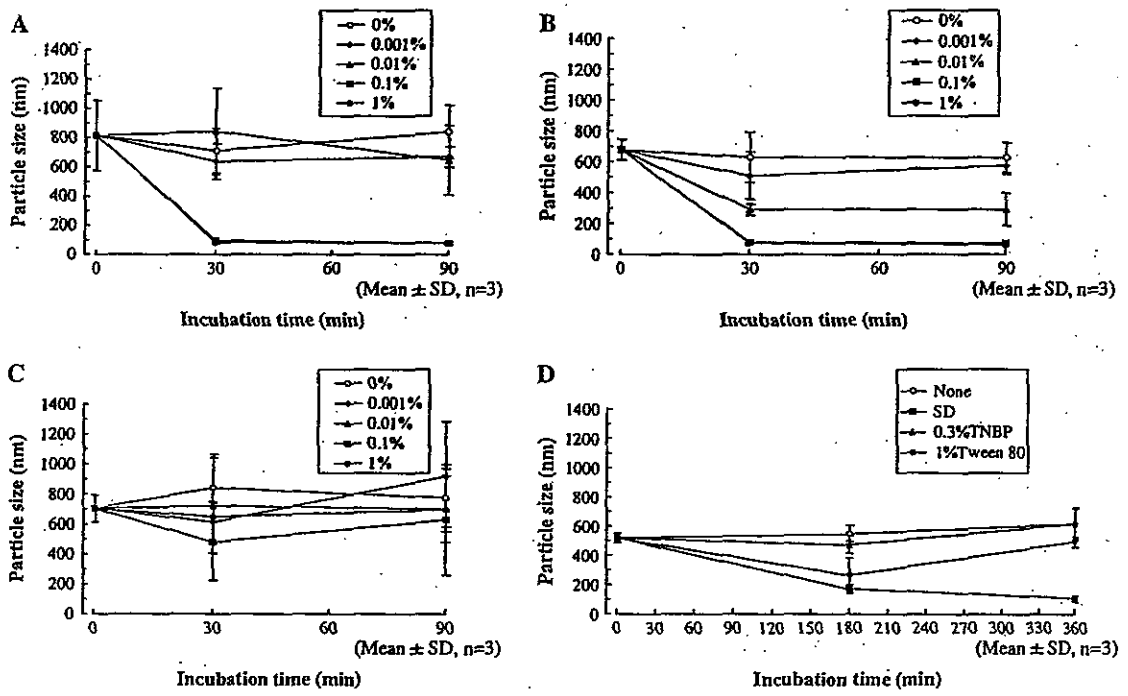

Fig. 1. Change of particle size in normal MF following treatment with various detergents. To normal MF, sarkosyl (A), lysolecithin (B), or Triton X-100 (C) was added to a final concentration of 1%, 0.1%, 0.01%, and 0.001%, respectively. The change in the average particle size was then monitored at room temperature for 90 min. In addition, TNBP or Tween 80 was added to normal MF to a final concentration of 0.3% and 1%, respectively, either alone, or in combination ("SD treatment"). The change in the average particle size was then monitored at 37 °C for 6 h (D).

with 0.1% sarkosyl, 0.1% lysolecithin, "SD treatment", or "super-sonication". The use of detergent or "SD treatment", in combination with "super-sonication", was also shown to effectively reduce the average particle size in normal MF preparations, to comparable levels to the individual treatments alone (data not shown). "Super-sonication" has an advantage over the other treatments in that it can minimize the change of composition of samples taken from the manufacturing process, as it does not require the addition of reagent(s) to the normal MF. For this reason, "super-sonication" is considered to be a useful approach for the treatment of 263K MF for process evaluation. "SD treatment", although slightly less effective, is used in many manufacturing processes, and may therefore be useful alone, or in combination with "super-sonication", for the process evaluation of products whose manufacturing process includes an "SD treatment" step. These approaches, alone or in combination, may also be useful to prevent the clogging of filters that can occur during spiking studies.

3.2. Infectivity of PrP^{Sc} in 263K MF and influence of 263K MF preparation methods on infectivity

The effect of "super-sonication" and "SD treatment" on the infectivity of 263K MF was studied. Infectious titers of

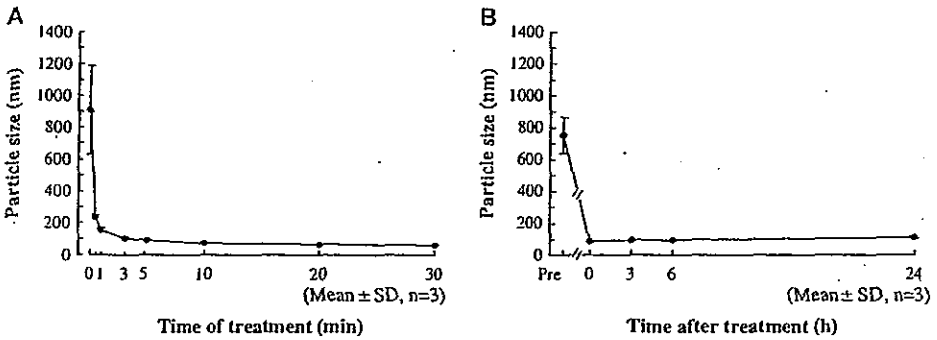

Fig. 2. Change of particle size in normal MF following intense sonication ("super-sonication"). Normal MF in a test tube equipped with a resonance chip (20 kHz, 200 W) was sonicated for 1 min in an ice bath. After 1 min, the sonication step was repeated. The change in average particle size was monitored during 30 cycles of sonication (A). After 10 cycles of sonication ("super-sonication"), normal MF was held at room temperature for 24 h, and the change in particle size was monitored (B).

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263K MF, "super-sonicated" 263K MF, and 263K MF subjected to "SD treatment", ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended with thrombin starting material, "super-sonicated", and 220 nm-filtered, were determined using a hamster bioassay. The results are summarized in Table 1.

The titers of two independent batches of 263K MF treated by "super-sonication" were 6.0 and 5.3 $\log_{10}$ LD_{50}/ml, respectively. The titer of the "non-super-sonicated"263K MF used to generate one of these stocks was 5.7 $\log_{10}$ LD_{50}/ml. These results suggest that "super-sonication" does not influence the infectivity of 263K MF. The titer of the 263K MF subjected to "SD treatment", ultracentrifuged at 141,000 × *g* for 60 min at 4 °C, resuspended with the thrombin starting material, "super-sonicated", and 220 nm-filtered, was 6.9 $\log_{10}$ LD_{50}/ml, which was approximately 1 log higher than that of the corresponding stock treated by "super-sonication" alone. Whether this difference is significant is unclear. The process to generate the "SD-treated" spike materials included an ultracentrifugation step. We were therefore concerned about recovery of infectivity following centrifugation, as the particle size of 263K MF was highly reduced by the "SD treatment" step. However, these results suggested that the recovery of infectious particles following ultracentrifugation was satisfactory.

Although it is possible that use of a 200 day bioassay may under-estimate the infectious titer of the 263K MF stocks, the use of a relatively short duration bioassay is considered unlikely to affect the main conclusions drawn. At least the last two dilution groups tested showed no animals with evidence of scrapie infection in all four titrations, and only three animals in the study (one in each of three separate titrations) developed clinical symptoms necessitating euthanasia later than day 131 (euthanized on days 160, 183 and 183, respectively), suggesting the titers obtained for all the stocks are close to end-point (data not shown). In addition, as others have demonstrated that treatment with detergent, and exposure to treatments that result in inactivation of the scrapie agent, such as heat or NaOH, may result in extended incubation periods for clinical scrapie, if anything the results may under-estimate the relative titers of the treated stocks [17,18]. Therefore, the bioassay results support the conclusion that "super-sonication" of 263K MF stocks, with or without "SD treatment", does not appear to significantly reduce the infectious titer of the stock, and that these preparations are therefore suitable for use in prion clearance studies.

3.3. Removal of PrP^{Sc} by various filters

To determine whether "super-sonication" influenced the $\log_{10}$ reduction observed for PrP^{Sc} following filtration under defined conditions, "super-sonicated" or "non-super-sonicated" stocks of 263K MF were diluted in PBS, and then filtered through 220 nm, 100 nm, P-75N, P-35N, and P-15N filters. Samples were analyzed by WB. The results are summarized in Table 2. The use of "super-sonicated" 263K MF appeared to result in lower $\log_{10}$ reduction values, supporting the idea that "super-sonication" of 263K MF produces a more severe challenge for a filter step. An approximately 5-fold higher $\log_{10}$ reduction factor was observed for "non-super-sonicated" stocks, for the 100 nm and P-75N filters, for both stocks tested. No significant loss of PrP^{Sc} was observed with either spiking material with 220 nm filtration, and no PrP^{Sc} was detected in the filtrates following P-35N and P-15N filtration.

Previously, we have observed some removal of PrP^{Sc} in some lots of "non-super-sonicated" 263K MF by 220 nm filtration. Strict control of the methodology used to generate the 263K MF stocks appeared to prevent this, suggesting that the method of preparing the 263K MF itself may influence the particle size distribution (data not shown).

3.4. Removal of PrP^{Sc} by Planova filters in the presence of plasma preparations

Removal of PrP^{Sc} by P-15N, P-20N, and P-35N filters was evaluated in the presence of a number of different plasma preparations, under conditions designed to mimic the relevant manufacturing process. The design of the experiments was similar to that of virus clearance studies. Samples were analyzed by WB, and the $\log_{10}$ reduction factor (LRF) was calculated for each filter step. The results are shown in Table 3.

Under all the experimental conditions tested, PrP^{Sc} was not detected by WB after filtration through P-15N. The LRF values were $\geq$2.8. In contrast, PrP^{Sc} was detected by WB in samples following filtration through P-20N and P-35N filters, in three out of the four processes tested, giving LRF values in the order of 2 logs. In one study, P-35N/haptoglobin, using "super-sonicated" 263K MF, PrP^{Sc} was not detected in the filtrate. However, the sensitivity of this study was low, giving a LRF of $\geq$1.4, and therefore the robustness of this filtration process was not evaluated. In the initial studies (Table 2), PrP^{Sc} was not detected in the fractions after P-35N filtration of either "super-sonicated" or "non-super-sonicated" 263K MF in PBS, resulting in log reduction factors in the order of 3 logs. The variance in the results obtained for these filters could be due to a combination of factors, including how the scrapie spike material was prepared, the composition of the starting material, and the precise filtration conditions used.

3.5. Removal of prion infectivity by Planova filters in the presence of plasma preparations

P-15N filtration was shown in these studies to be able to remove PrP^{Sc} to levels below the detection limit of the WB assays used, regardless of the method used to prepare the spike material, the composition of the start material, or the filtration conditions. However, a bioassay study for samples generated in a P-15N/AT study using 220 nm-filtered "super-sonicated" 263K MF, demonstrated that infectivity was recovered following filtration, as clinical signs appeared in all hamsters inoculated with the filtrate, and analysis of hamster brain material confirmed the clinical results. PrP^{Sc} was detected in the brain homogenates from all clinically positive hamsters by WB, and scrapie-associated lesions were observed in all the

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Table 4
Scrapie infectivity in samples generated during the P-15N/AT study

	Before filtration			Filtrate		
	Animal number			Animal number		
	1	2	3	1	2	3
Appearance of clinical signs (day euthanized)	87	87	87	94	143	105
PrP^{Sc} in brain by WB3	Detected	Detected	Detected	Detected	Detected	Detected
Lesions by histopathology	+ve	+ve	+ve	+ve	+ve	+ve
Medulla (oblongata)	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P
Cerebellum (cortex)	D	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P
Midbrain	D,P	D,V,P	V,P	D,P	D,P	D,V,P
Hypothalamus	D,P	D,V,P	D,P	D,V,P	D,P	D,P
Thalamus	D,P	D,V,P	D,P	D,P	D,P	D,P
Hippocampus	NR	D,V	D	D	D,V,P	D,V
Paraterminal body	D,P	D,P	D,P	NR	D,V,P	P
Cerebral cortex (posterior midline)	D,P	D,P	D,P	D,P	D,V,P	D,V,P
Cerebral cortex (anterior midline)	D,P	D,V,P	D,V,P	D,V,P	D,V,P	D,V,P

Abbreviations used: +ve, scrapie positive; NR, no remarkable change; D, degeneration of nerve cell; V, vacuolation; P, proliferation of glial cell.

corresponding hamster brain material on histopathological observation (Table 4). Typical nerve lesions are shown in Fig. 3. Thus, P-15N filtration did not result in the complete removal of infectivity, for this process step.

4. Discussion

In this study, we have investigated the capacity of P-35N, P-20N and P-15N filters to remove the 263K scrapie prion protein, PrP^{Sc}, under the conditions used for the manufacture of four different plasma-derived products, using spike preparations designed to present a serious challenge to the filters.

Validation studies to evaluate the capacity of manufacturing processes to remove potential contaminants, including prions, are required for biological or biopharmaceutical products intended for human use. When designing these studies, a worst-case challenge should be used wherever possible, to minimize the risk of over-estimating the capacity of the process to remove such contaminants. Virus removal filters (or nanofilters) are designed to remove contaminants predominantly on the basis of size. The worst-case challenge for such steps should therefore be a preparation containing the smallest possible form of the infectious agent.

TSE clearance studies provide a particular challenge in that the nature of the infectious agent is still uncertain, and the forms of infectious agent present in plasma, and/or during the different stages of a manufacturing process, are not clearly understood. The causative agent of TSE diseases is believed to be strongly associated with, if not solely composed of, the disease-associated prion protein, PrP^{Sc}. Normal cellular PrP is a membrane-bound glycoprotein, which associates with membranes through a glycosylphosphatidylinositol (GPI) anchor. Prion infectivity is associated with heterogeneous particles, including membranes, liposomes and protein aggregates, so called prion rods. Therefore, methods which result in solubilization of membrane proteins, or dispersal of membrane fragments, vesicles and/or protein aggregates, may be expected to reduce the size of particles associated with prion infectivity.

Treatment of MF preparations derived from brains of uninfected (normal) hamsters with either detergent (0.1% lysolecithin or 0.1% sarkosyl) or extensive sonication ("supersonication") resulted in a rapid reduction in the average particle size, to approximately 100 nm. SD treatment (1% Tween 80 and 0.3% TNBP for 6 h) also resulted in a reduction in particle size, although this was slower and less effective, reducing the average particle size to the order of 200 nm.

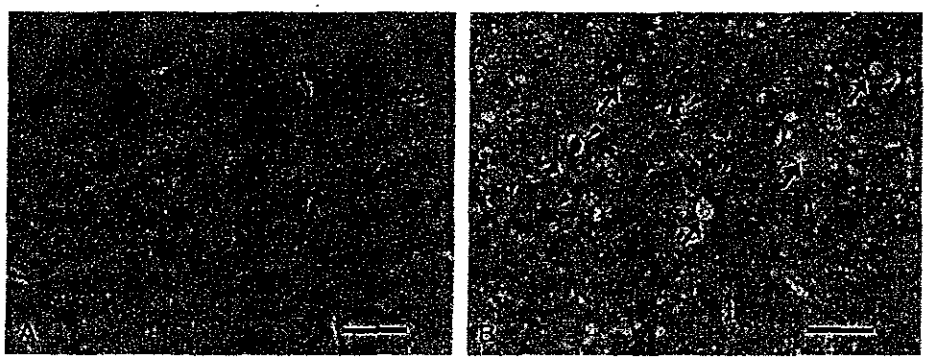

Fig. 3. Typical nerve lesions in the hippocampus of a hamster brain, taken from an animal inoculated with a P-15N-filtered sample (B), in comparison with the corresponding region from an uninfected animal (A). Arrows, vacuolation; Arrowheads, degeneration of nerve cells; scale bar = 50 μm; HE staining used.

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"Super-sonication" has the advantage that it is a physical disruption process, and does not alter the chemical composition of the spike material, thus minimizing changes to the start material used for nanofiltration. SD treatment is included in many manufacturing processes for plasma-derived products, and therefore, although not as effective as "super-sonication", use of this treatment might be expected to result in a spike material more closely mimicking the form of infectious prion present in the relevant start material during the manufacturing process. Use of these treatments alone or in combination may therefore be useful in reducing the size of infectious particles present in TSE spike materials for prion clearance studies.

The effect of the above treatments was studied using normal MF, as the facility was unable to handle infectious TSE materials. Although some care should be taken in extrapolating these results to TSE-infected brain material, "super-sonication" of 263K MF preparations appeared to reduce the removal of PrP^{Sc} following filtration, while detergent-treated spike preparations have previously been shown to present a more significant challenge to nanofiltration steps than untreated preparations ([9,10] and own unpublished observations). Furthermore, "super-sonication", with or without SD treatment, does not appear to reduce the level of infectivity present within the 263K MF, supporting the use of such preparations for prion clearance studies.

Using 263K MF treated with 0.1% sarkosyl, "super-sonication" or SD plus "super-sonication", we investigated the prion removal capacity of P-15N, P-20N and P-35N filters in the manufacturing processes used for four different plasma products. The results obtained suggest that both the composition of the materials to be filtered and the prion load influences the removal of prions. PrP^{Sc} was recovered in the filtrate fraction from three out of the four processing steps performed for P-20N and P-35N. In contrast, under all conditions tested, P-15N filtration resulted in removal of PrP^{Sc} to below the limit of detection of the Western blot assays used. Thus, P-15N would appear to be a more robust method for the removal of prions, reproducibly giving LRF in the order of 3 logs, under the conditions tested. In practice, however, it is not feasible to incorporate P-15N filtration into the manufacturing process for all plasma derivatives. From the results shown in Table 2, it may also be possible to optimize processing conditions to allow effective removal of PrP^{Sc} using P-20N or P-35N filters.

WB assays were used to monitor the partitioning of PrP^{Sc} during the nanofiltration processes. WB assays are semi-quantitative and serve to provide an indication of the relative levels of PrP^{Sc} present in different samples. However, there are limitations to the sensitivity of available WB assays, and these assays provide only an indirect measure of infectivity. Therefore, to confirm that removal of PrP^{Sc} does reflect removal of infectivity, bioassays need to be performed.

Although PrP^{Sc} was not detected in any of the P-15N filtered samples by WB assay, infectivity was recovered in a filtrate fraction tested by bioassay for one process run. Foster also noted that infectivity was detected in a filtrate fraction after P-15N filtration ([8] reported as personal communication; data not shown). Thus, even with P-15N, depending on the processing conditions, there may be incomplete removal of prion contaminants.

Although infectivity was detected in the filtrate fraction from the one process step studied, longer and more variable incubation periods were observed in the animals inoculated with the filtrate sample (Table 4), suggesting a lower prion titer following filtration. However, it was not possible to estimate the relative levels of prion infectivity present in the input and filtrate samples, as no data was available to correlate incubation periods and prion titers for this study. Based on the titers typically observed for 263K MF stocks, the bioassay used could theoretically detect reductions in prion infectivity in the order of 4 logs for this process step. Detection of infectivity in the filtrate fraction by bioassay is therefore not necessarily incompatible with the WB results obtained (LRF $\geq$2.8 logs), and may simply reflect a difference in sensitivity between the two assays used.

As discussed above, uncertainties about the nature of the infectious agent in plasma, and during the manufacturing process, raise concerns about the design and interpretation of prion clearance studies. No single spike preparation is likely to contain all potential forms of the infectious agent. Infectivity is associated with membranes and protein aggregates. In addition, it has recently been shown that the GPI anchor is not required for infectivity, suggesting that endogenous proteolytic release of PrP^{Sc} from cell surfaces may also contribute to the spread of the infectious agent *in vivo* [19,20]. Whether significant levels of infectivity in human plasma are associated with GPI-anchorless prion protein is not yet clear. These different forms of infectivity, with different biophysical properties, could show different partitioning properties through the same manufacturing process [7]. Furthermore, different forms of the agent may differ in their level of infectivity. For example, it was recently reported that particles in the order of 17–27 nm appeared to have the highest relative level of infectivity, in comparison to levels of PrP^{Sc} [21]. Therefore, a better understanding of the nature and forms of the infectious agent is essential to allow the design of more accurate models for prion clearance studies, and a more confident evaluation of the safety of manufacturing processes with respect to potential TSE contamination.

In summary, we used methods intended to reduce the size of particles present within MF preparations in an effort to present a worst-case (smallest) prion challenge during nanofiltration. Using such preparations, P-15N filtration consistently reduced the level of PrP^{Sc} to below the limits of detection of the Western blot assays used, suggesting that this process step is effective for the removal of prions. However, data from a single process step studied suggested that infectivity could be recovered following P-15N filtration, and thus even P-15N filtration may not result in complete removal of prions, at least when used under some conditions.

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Planova filters. Some of the data presented in this study has been summarized in a recent review [22].

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研究報告の概要

英国血漿由来の第 XI 因子製剤が、1989-2000 年の間に米国内の様々なサイトにおいて新薬臨床(IND)試験に参加した 50 人以下と推定される少数の患者の治療に使用された。使用された英国製第 XI 因子製剤はいずれも、後で vCJD と診断されたドナーからの血漿を含む血漿プールからは製造されていなかった。しかしながら、英国の血漿提供者を含む英国人集団は、リスク評価は広範囲に変わるとはいえ、BSE への食物連鎖曝露により、米国人集団よりも vCJD リスクは非常に高い。
100 万人当り 4 人まで vCJD 感染者が存在するとする低い方の症例ベースの感染者推定値では、平均して約 1.6%のみの血漿プールが vCJD 病原体を含んでいる可能性があると予測できる。一方、一つの組織の調査に基づく 4,225 人に 1 人の英国人に vCJD 感染者がいるとする高い方の感染者推定値では、50%の血漿プールに vCJD に汚染されたヒト由来の血漿ドネーションが含まれていた可能性があると予測された。そして、げっ歯類動物モデルの実験結果をもとにした患者 1 人が第 XI 因子の治療当り受ける vCJD 感染リスクは、原料に用いた血漿ドナーに低い方の感染者推定値及び高い方の感染者推定値を適用し計算すると、各々①シナリオ 1(投与量が 3,000u の場合)：1/643、1/17、②シナリオ 2(投与量が 9,000u の場合)：1/214、1/5.6、③シナリオ 3(投与量が 15,000u の場合)：1/129、1/3.4 であった。
このモデルの結果は、vCJD 病原体の暴露があった可能性があり、潜在的感染リスクがあることを示唆しているものの、英国血漿から製造された第 XI 因子製剤を 2000 年まで使用した個々の患者に対して、本当に意味があり正確な vCJD リスクの推定値を提供することは不可能である。疫学的モデルを元にした英国 vCJD 感染率推定値が正しいと仮定すると、曝露の可能性及び感染リスクはかなり低い。また、リスクが低いにもかかわらず、リスク評価に使用できる情報の不足と不確かさから、実際のリスク評価は難しい。なお、このリスク評価の結果を考慮する際、血漿由来製剤の投与を受けた患者において、これまで世界中で一件も vCJD の症例が報告されたことがないことに注意を向けることが大切である。この中には、長い期間に亘って、英国の血漿ドネーションから製造された他の製剤を大量に投与された患者に加えて本製剤を投与された患者が存在する。
このリスク評価モデルは、製造工程の vCJD 病原体のクリアランス、ヒトの製剤使用量、脳内接種に対して静注ルートの効率及び英国ドナーの vCJD 感染陽性率がリスクに影響する最も重要な要因であると指摘している。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
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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
英国血漿由来の第 XI 凝固因子製剤の投与を受けた米国レシピエントにおける vCJD 病原体に対する FDA のリスク評価案である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

FDA DRAFT Risk Assessment: Potential Exposure to the variant Creutzfeldt-Jakob Disease Agent in United States Recipients of Factor XI Coagulation Product Manufactured in the United Kingdom

November 7, 2006

Center for Biologics Evaluation and Research
US Food and Drug Administration

CONTRIBUTORS

Center for Biologics Evaluation and Research

Office of Biostatistics and Epidemiology
Steven Anderson
Hong Yang

Office of Blood Research and Review
Jay Epstein
Mark Weinstein
Jonathan Goldsmith
David Asher
Dorothy Scott
Rolf Taffs

Office of the Center Director
Jesse L. Goodman
Karen Midthun
Diane Maloney

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EXECUTIVE SUMMARY

Variant Creutzfeldt-Jakob disease (vCJD) is a fatal neurodegenerative disease attributed to human infection with the agent of bovine spongiform encephalopathy (BSE) and is most often transmitted by the consumption of beef products from infected cattle. Cases of vCJD were first reported in humans in the UK in 1996, and as of August 2006, 195 cases have been reported worldwide, with 162 cases in the UK. Since December 2003, there have also been three reports in the United Kingdom (UK) of probable vCJD transmission by red blood cell transfusions. The donors were healthy at the time of donation, but later developed vCJD. Of the three red blood cell recipients who probably became infected with the vCJD agent after transfusion, two developed vCJD and died from the disease. The third died of an unrelated illness

The probable transmission of vCJD via red blood cell transfusions raised the possibility that plasma derivatives might also pose a risk of vCJD transmission, although there have as of yet been no reported cases of vCJD in any recipients of plasma derivatives in the UK, where the risk is considered greatest, or elsewhere. UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors, about the potential for risk of vCJD from these products. These products included coagulation factors VIII, IX, and XI, as well as antithrombin III, and intravenous immune globulins. The derivatives of concern were manufactured from plasma collected from UK donors from 1980 through 1997. In 1998 UK manufacturers stopped using UK plasma. The last expiry date for any of the UK products was in 2001.

Problem: Some Factor XI (FXI) made from UK plasma was used between 1989 and 2000 to treat a small number of patients, estimated to be 50 or fewer, who participated in several Investigational New Drug (IND) studies at various sites in the US. No FXI product used in the US was manufactured from a pool containing plasma from a donor diagnosed with vCJD (that is, there were no known "implicated" lots). However, the UK population, including UK plasma donors, is at a considerably higher risk for vCJD than the US population due to food chain exposure to BSE, although the estimates of risk vary widely.

Question addressed by risk assessment: *Given the probable recent transmission of vCJD via transfusion of red blood cells, what is the estimated range of potential risk to US recipients who received a human plasma derived FXI product manufactured from UK plasma?*

FDA presented the "Draft Risk Assessment: Potential Exposure to the vCJD Agent in United States Recipients of Factor XI Coagulation Product Manufactured in the United Kingdom" at the February 8, 2005 meeting of the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) for review and comment. The risk assessment computer model predicted that recipients of UK manufactured FXI may have potentially been exposed to the vCJD agent, and although the risk of possible vCJD infection could potentially be significant, the actual risk is highly uncertain and could be low. On October 31, 2005 FDA sought advice and discussion on several risk assessment model inputs for plasma derivatives and potential vCJD risks. FDA has incorporated comments and advice provided by the TSEAC at the February 8, 2005 and October 31, 2005 meetings, and by FDA staff and Special Government Employee peer reviewers to develop this revised risk assessment (revisions summarized in Table 1 of the Risk Assessment) of potential vCJD risks for US recipients of FXI manufactured from UK donor plasma.

Results from the Model

While the risk assessment suggests that the possibility of exposure to vCJD in FXI could be potentially significant the actual risk is highly uncertain. One important, highly uncertain parameter in driving the risk assessment results is what estimate is 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 and an estimate, based on the epidemic, 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 results from Clarke and Ghani (2005), yielded an estimate of approximately 4 vCJD cases per million. Running the model with this vCJD case prevalence estimate of 4 per million produces an estimate suggesting that, on average, there is a 1.6% likelihood that a plasma pool of 20,000 donations will contain at least one donation from a vCJD-infected individual. Therefore, on average, 98.4% 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 (below).

However, it is possible that the prevalence of vCJD in the UK is higher than the estimate based on the epidemiological model-based approach noted 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 prion protein. It yielded a much higher 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 or interpretations from the tissue samples are possible. It is also not known whether the staining of appendicial tissues used in this study is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation. However, while unconfirmed, the findings from this study provide a higher prevalence estimate and therefore should also be considered. 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 50% likelihood that a plasma pool containing 20,000 donations will contain at least one donation from an individual whose blood contains the vCJD agent. (see Sections III. A.1.a.iii and III. A. 1. b. below for a more complete discussion of some of the uncertainties in these prevalence estimates).

Table I – Mean Potential vCJD Risk per Person per FXI Treatment Scenario

		MODEL OUTPUT USING LOWER PREVALENCE ESTIMATE *vCJD Case Prevalence from epidemiological modeling ~4 per million (Clark and Ghani, 2005)*	*MODEL OUTPUT USING HIGHER PREVALENCE ESTIMATE* *vCJD Infection estimate from tissue surveillance study 1 in 4,225 (Hilton et al 2004)*
Scenario	**Quantity* FXI Utilized**	**Mean potential vCJD risk per person **** (5^{th}, 95^{th} perc)	**Mean potential vCJD risk** per person** (5^{th}, 95^{th} perc)
Scenario 1: Treatment 3,000u	3,000 u	1 in 643 (0 - 0)	1 in 17 (0 – 1 in 3.5)
Scenario 2: Treatment 9,000 u	9,000 u	1 in 214 (0 - 0)	1 in 5.6 (0 – 1 in 1.2)
Scenario 3: Treatment 15,000 u	15,000 u	1 in 129 (0 - 0)	1 in 3.4 (0 – 1 in 1)

*u - represents units of FXI

**Mean potential vCJD risk per treatment scenario – the risk of potential vCJD infection based on animal model dose-response information.

Results from the model are presented in **Table I**. The mean potential vCJD risk per person per treatment scenario is based on data derived from rodent animal models. Although we assume that results from the animal model are similar for humans, it is uncertain whether this is the case.

Three scenarios were modeled to depict various plausible levels of utilization of FXI. FXI doses in the literature typically range from 20 – 50 u/kg, and total doses as high as 15,000 u/patient have been administered as the sum of several doses during the postoperative setting, over a period of days. Therefore, Scenario 1 involves the treatment of a 60 kg individual with 20 – 50 u/kg, or a total of 3,000 units given to restore FXI levels to normal. Scenario 2 and Scenario 3 assume a treatment regimen consisting of 9,000 units, and 15,000 units of FXI, respectively.

The model estimates risk ranges from a high in Scenario 3, using the high prevalence estimate, with a mean estimated per person (per treatment course) risk of vCJD infection of 1 in 3.4. The lower end of the range of risk is illustrated in Scenario 1, at the lower prevalence estimate which is based on the current epidemic of disease, with a mean estimated per person (per treatment course) risk of vCJD infection of 1 in 643. It can be seen that in this model that the prevalence estimate may determine whether anyone receiving this product may or may not have been likely to be

exposed to a vCJD risk. It is simply not known at present which prevalence estimate may more accurately reflect the true prevalence of vCJD in the UK.

Readers may notice that the 5th and 95th percentile intervals for all of the model outputs using the lower prevalence estimate are from 0 to 0 meaning that the chance of an infected donor donating to a plasma pool would be an infrequent event. This means that at least ninety five percent of the time the model estimates the risk to be zero because vCJD agent was not present in FXI product vials used during treatment. However, the model predicts that 1.6% of the time the exposure to vCJD may be greater than zero. Although the model suggests that exposure of FXI recipients may have occurred, it is not possible at this time to determine if exposure did in fact occur because of the large variability and uncertainties in the data used in the model.

Conclusions

No UK-manufactured FXI product used in the US under IND from 1989 to 2000 was manufactured from "implicated" plasma pools that were known to have contained plasma from a donor later diagnosed with vCJD. With use of the lower, case-based, prevalence estimate for vCJD of ~ 4 vCJD cases per million, the model predicts that only about 1.6% of plasma pools, on average, might contain the vCJD agent. However, if the higher prevalence estimate, based on a single tissue study, for vCJD in the UK of 1/4,225 is used as a possible higher estimate of the actual vCJD prevalence, the model predicts that most (50%) plasma pools used to manufacture FXI until 1998 could have contained a plasma donation from a person infected with vCJD. Although results of the model suggest there may have been exposure to the vCJD agent, and there could be a potential risk of infection, it is not possible to provide a truly meaningful or accurate estimate of the vCJD risk to individual patients who used FXI manufactured from UK plasma through 2000. Assuming that the UK vCJD prevalence estimate generated from epidemiological modeling is correct, the possibility of exposure and the risk of infection would be considerably lower. Again, although the risk may be low, it is difficult to actually estimate the risk with confidence given the multiple unknowns and uncertainties of the available data used in the model (see sections III.A.1. and IV.D. for discussion). In considering which prevalence estimate is more likely to be correct and in considering in general the risk estimates from the model, it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products. This includes patients receiving this product as well as patients receiving large amounts of other products manufactured from UK plasma donations over a long period of time. 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, efficiency of the i.v. versus the i.c. route of exposure, and the vCJD prevalence in the UK donor population.

RISK ASSESSMENT

BACKGROUND

FXI

FXI is a clotting factor present in blood plasma that plays a role in the very early stages of the blood coagulation pathway. FXI is normally present in human plasma at concentrations of 50-70 u/dl.

FXI deficiency is a rare bleeding disorder first described in the 1950s. Unlike hemophilia A or B, it is an autosomal bleeding disorder that affects both genders equally. Generally, bleeding in patients with FXI deficiency is less severe than with hemophilia A or B and does not usually involve joints or muscles, or spontaneous bleeding in those areas (http://www.hemophilia.org/bdi/bdi_types9.htm). FXI deficiency is usually categorized as severe or partial. Those with severe deficiency have FXI levels below 15 u/dl and are at high risk of excessive bleeding if injured, or after surgery or dental extractions. Medical intervention that brings FXI levels to the 50 u/dl to 70 u/dl range is recommended (BPL, 2001) prior to surgical procedures on severely deficient patients.

FXI, manufactured from UK donor plasma collected through 1997, was used by a small group of patients in several IND studies in the US between 1989 and 2000, and this risk assessment estimates the potential exposure to vCJD agent via that product. Currently, in the United States where there is no commercially licensed FXI product; clinicians typically utilize Fresh Frozen Plasma from US donors, and/or antifibrinolytic agents for therapy.

I. 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. To date, no epidemiological evidence suggests that vCJD has been transmitted by plasma derivative products.

A new human variant of Creutzfeldt-Jakob disease (vCJD) was first described in the UK in 1996. As of August 2006, 195 cases worldwide have been reported including 162 in the UK (United Kingdom National CJD Surveillance Unit, August 2006).

Both vCJD and BSE belong to a class of diseases known as transmissible spongiform encephalopathies (TSEs). The leading theory concerning the agent responsible for infection is that of a proteinaceous infectious agent, or "prion," that originates in the misfolding of a ubiquitous prion protein (PrP) normally expressed in many cells. The altered PrP, which is now suggested to be designated as PrP^{TSE}, (World Health Organization Guidelines, 2006, also known as PrP^{res}, or PrP^{Sc}) is highly stable and resistant to degradation by high heat and many chemical treatments commonly used to denature proteins. The incubation from the time of exposure to TSEs to the

development of symptoms of disease is long. For example, the mean incubation period for BSE in cattle (interval between first exposure to contaminated feed and onset of illness) has been estimated at about 5 years, and that for food-borne vCJD is estimated to exceed 10 years. Individuals become symptomatic with vCJD only in the last few months of life, making early detection very difficult. Confirmatory diagnosis of vCJD requires postmortem examination of brain tissue. However abnormal prion protein has been detected in some antemortem tonsil biopsies early in clinical illness, and in an archived appendix sample from an asymptomatic individuals several months prior to the onset of symptoms (Hilton *et al* 1998). There are currently no validated tests available to detect the vCJD infectious agent in early stages of infection or to detect the presence of TSE agents in blood.

I.A. 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) and naturally infected with scrapie (Hunter *et al* 2002), and experimentally infected rodents (hamsters with scrapie and mice with a human TSE) (Rohwer 2004, 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.

I. B. Transfusion transmission of vCJD in the UK

In December 2003 the UK government announced that vCJD had likely been transmitted to a 69 year-old patient via blood transfusion. The patient had received non-leukoreduced red blood cells in 1996 from a donor who died three years later of vCJD. This first report was followed by the announcement in July 2004 of another probable case of transfusion-transmitted vCJD. The patient died of a ruptured aortic aneurysm without clinical evidence of vCJD, but postmortem testing detected PrP^{TSE} in spleen tissue and cervical lymph node. In February 2006 a third case of probable transfusion transmitted vCJD was reported in the UK in a 31 year-old male; the patient had received a transfusion eight years earlier from a donor who died of vCJD 20 months after donation. None of the donors were known to have had vCJD at the time of donation.

It is possible that dietary exposure may have been responsible for some or all of the three cases that were reported after red blood cell transfusions. However, the probabilities for occurrence of either a single, or, particularly, two or three such events are small. As Llewelyn *et al* (2004) pointed out in their publication discussing the first presumed transfusion-transmitted case "the age of the patient was well beyond that of most vCJD cases, and the chance of observing a case of vCJD in a recipient in the absence of transfusion transmitted infection is about 1 in 15,000 to 1 in 30,000." The combined probability that the first two transfusion cases both acquired infection

from food, identified in two elderly patients in a small cohort of transfusion recipients in an age group underrepresented among vCJD cases, is remote.

The presumptive transmission of vCJD via red blood cell transfusion in the UK raises the possibility that plasma derivatives may pose a risk, based on the finding in animal models that plasma contains the infectious agent when blood is infectious. The UK authorities have notified physicians in the UK and their patients who received plasma derivatives made from plasma from UK donors about the potential for risk of vCJD from these products. These plasma derivative products included coagulation factors, as well as antithrombin III, and intravenous immune globulins. The derivatives of concern were manufactured from plasma of UK donors between 1980 and late in 1999, when--consistent with a decision announced in 1998—UK manufacturers stopped using UK plasma. The last expiry date for any of the UK products was in 2001. To date, no cases of vCJD have been reported in any recipients of plasma derivatives, either in the UK, where the risk is considered greatest, or elsewhere, including in patients who have received human plasma-derived coagulation products from implicated lots (i.e. lots that were later found to contain donations from people who later developed vCJD) made in the UK.

This risk assessment examines the possible exposure to vCJD agent and the risk of vCJD infection for FXI manufactured from UK plasma between 1989 and 1997 and used by a small group of patients in several IND studies in the US between 1989 and 2000.

II. HAZARD CHARACTERIZATION

The hazard characterization component (also known as dose-response) relates the information in the exposure assessment, which determines the dose, to the adverse consequence(s) such as infection, illness, etc., at the individual, subpopulation, or population level. Determining dose-response relationships can be difficult to accomplish because data are limited, especially exposure and outcome data for humans. Other factors such as characteristics of the hazard (e.g. strain, chemical make-up, etc.), route of introduction, and 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.

The rodent animal models drawn on for this risk assessment on vCJD risks use other TSE agents such as the scrapie agent – hamster model used by Rohwer (2004) and the CJD agent-mouse model used by Brown (1998, 1999). Brown (1998, 1999) and Rohwer (2004) use the metric of infectious unit (IU), which is the minimal amount of any inoculum required to initiate an infection in 100% of the rodent population using the intracerebral (i.c.) route of introduction. The FDA model assumes that this infectious unit is equivalent to two ID_{50}s which assumes the two metrics are linearly related, further adding to the uncertainty of the model.

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, such as is seen with many other pathogens such as viruses or bacteria, for which infection requires exposure to at least one, and often more, units of the infectious agent. Furthermore, it is not known whether the effects of small multiple exposures

over a period of time are cumulative and may result in the possibility of infection and disease equivalent to a single, larger exposure (e.g., via intracerebral injection in laboratory animals). Some risk assessments have made assumptions concerning the exposure and dose for TSE agent that lead 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 Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) (2005), and consistent with suggestive data from studies of TSE agents in animal models (Diringer *et al* 1998, Jacquemot *et al* 2005), FDA also assumes that exposure to vCJD ID_{50} is cumulative over a one year period. The ID_{50} is the common metric used to quantify the infectivity of TSEs. One ID_{50} is defined as the amount of infectious material or tissue that is necessary to initiate infection in 50% of the treated population. The route of exposure to TSE infectious material influences the efficiency of transmission of the disease. Based on advice provided to FDA by the TSEAC (October 31, 2005) the model assumes that transmission via the intravenous (i.v.) route is between 1 and 10 times less efficient than the transmission via 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 FXI 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 have a probability of initiating infection in an individual, albeit the probability of infection would likely be low at low levels of exposure. The model further assumes that in such a case exposure to 1 ID_{50} would suggest a 50% probability of infection, exposure to 0.1 ID_{50} would suggest a 5% probability of infection, and so on. However, given the lack of information and high degree of uncertainty on the dose-response relationship because of the limited data available for TSE agents it is plausible that low level exposures, even on a chronic basis, may not attain a threshold or minimum quantity of agent necessary to initiate infection in humans. Again, FDA makes a conservative assumption that low-level exposure(s) over the period of one year to any quantity of vCJD agent could potentially lead to infection and that there is not a minimum dose necessary to initiate infection.

There are considerable uncertainties in determining the correct form for the vCJD-human dose-response model. For instance, the nature of the dose-response line, its slope, or whether it is more accurately described using a dose-response curve is uncertain because animal data are so limited and human data are not available. The FDA risk assessment estimates the potential individual risk of infection and assumes that a linear interpretation of the rodent model accurately reflects the pathology and progression of vCJD infection and disease in humans, but it may not. Furthermore, exposure to the vCJD agent may not necessarily lead to infection, and vCJD infection may not necessarily produce symptomatic vCJD disease or illness in an individual or population.

III. EXPOSURE ASSESSMENT

Exposure assessment evaluates the routes of exposure to a hazard, the probability that exposure occurs and the amount of a hazardous agent to which a person or population may be exposed. This exposure assessment specifically addresses exposure to the vCJD agent that may have been present

in FXI manufactured in the UK and administered to US patients during clinical studies under several IND applications. The administration of FXI, and thus the route of exposure, is intravenous and used in the clinical prophylactic treatment of individuals prior to surgery and after surgery to control bleeding.

Pools consisting of 20,000 or more plasma donations collected from UK plasma donors were used as the starting material from which FXI was purified. Because of the number of donations per plasma pool and the prevalence of vCJD in the UK population, it is possible that some plasma pools may have contained one or more plasma donations from asymptomatic donors unknowingly infected with vCJD.

Overview of Model

Figure 1 depicts the major elements and some of the types of input data and information used in the FDA FXI – vCJD risk assessment. Module A (vCJD ID_{50} per plasma pool) uses two different estimates of UK vCJD prevalence. The first is a vCJD case prevalence based on epidemiological modeling of actual reported cases in the UK and an estimate of future vCJD cases (Clarke and Ghani, 2005), which yielded an estimate of approximately 4 vCJD cases per million. 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 are described in section A.1.a. The second is a vCJD infection prevalence based on a tissue surveillance study by Hilton *et al* (2004), which yielded an estimate of 1 in 4,225 (237 infections per million) which represents a possible higher prevalence scenario. However, there are limitations to using the Hilton et al tissue surveillance study in estimating vCJD prevalence and those are described in section A.1.b.

After accounting for the age distribution and/or incubation period of possible vCJD cases, these prevalence estimates are used to predict the number of vCJD donations that could be present in a plasma pool of 20,000 donations. After adjusting for the relative efficiency of intravenous and intracerebral administration, the output of this module is an estimate of the vCJD i.v. ID_{50} per plasma pool. Module B approximates the reduction of vCJD agent during manufacturing. The model estimates a reduction of between 0 and 4 $\log_{10}$ (10,000 fold) in the amount of agent with a most likely level of reduction of 2 $\log_{10}$ (100 fold). The output of this module is an estimate of the ID_{50} per vial of FXI. Module C (Dose for Pre- / Post- surgical treatment) estimates utilization of FXI by patients. Estimates for potential exposure and potential vCJD infection risk were generated by the model for three possible clinical treatment scenarios.

Revisions of the February 8, 2005 FDA DRAFT Factor XI – vCJD risk assessment and model

FDA presented the first version of the risk assessment: "Draft Risk Assessment: Potential Exposure to the vCJD agent in United States recipients of Factor XI coagulation product manufactured in the United Kingdom" at the February 8, 2005 meeting of the TSEAC for review and comment. The risk assessment model predicted that recipients of UK manufactured FXI may have potentially been exposed to the vCJD agent. On October 31, 2005 FDA sought advice from the TSEAC and discussion on several risk assessment model inputs for plasma derivatives and potential vCJD risks. This newly updated, second iteration of the FDA Draft FXI vCJD risk assessment incorporates many of the comments and much of the advice provided by the TSEAC at the February 8, 2005 and October 31, 2005 meetings, and more recently by FDA staff and peer reviewers (revisions summarized in Table 1).

Figure 1. Model of Exposure Assessment

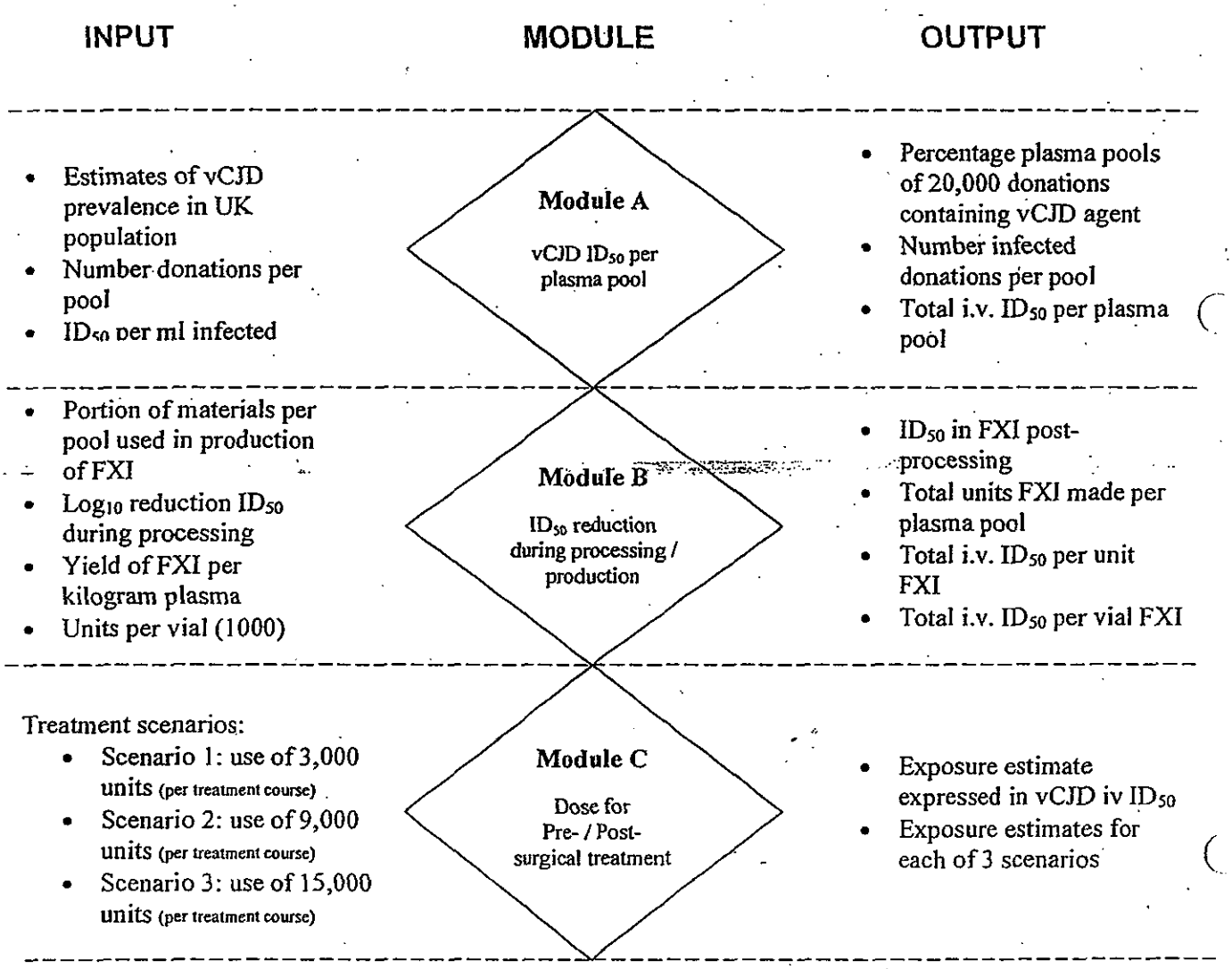


Table 1. FXI Model Input Changes from February 2005 to September 2006

	Model: Presented before TSEAC February 8, 2005 **Document:** Draft Risk Assessment: Potential Exposure to the vCJD Agent in United States Recipients of FXI Coagulation Product Manufactured in the United Kingdom	**Model: Revised September 2006** **Document:** FDA Draft Risk Assessment: Potential Exposure to the variant Creutzfeldt-Jakob Disease Agent in United States Recipients of FXI Coagulation Product Manufactured in the United Kingdom
Input Parameter Name and description	**Data and Parameters**	**Data and Parameters** TSEAC provided advice on vCJD and plasma derivatives at the October 31, 2005 meeting
UK vCJD Prevalence	Prevalence: **1 in 4,225** The UK vCJD Infection prevalence based on Surveillance study of tonsils /appendices samples from patients mostly 20 – 30 yrs of age *(Hilton et al 2004)*	Use two separate prevalence estimates: 1) HIGHER prevalence estimate – of UK vCJD Infection prevalence was based on Surveillance study tonsils/appendices of **1 in 4,225** *(Hilton et al 2004)* 2) LOWER prevalence estimate – vCJD Case prevalence estimate based on predictive modeling estimates - implies prevalence of **~4 vCJD infections per million** *(based on Clark and Ghani, 2005)*
Tissue surveillance or vCJD Infection prevalence: adjustment for donor age	No age adjustment was made. Prevalence based on surveillance study of tonsils /appendices samples from patients mostly 20 – 30 yrs of age was used.	Prevalence adjusted based on: 1) Age of donor population 2) Age information of reported vCJD cases
Time during incubation period when infectivity is present in blood	Infectivity assumed to be present **throughout the entire incubation period**	Infectivity assumed to be present only during **last half of incubation period**
Quantity of infectivity present in blood	Distribution: Triangular Minimum: **0.1** Most likely: **10** Maximum: **1,000** i.c ID50/ml	Distribution: LogNormal Minimum: **0.1** 5th percentile: **2** Median (50th perc): **12** 95th percentile: **30** Maximum: **1,000** i.c ID_{50}/ml
Adjustment for efficiency of transmission via intravenous (i.v.) versus intracerebral (i.c.) exposure	**0.5 -1**	**0.1 - 1**

III. A. Probability of donation containing vCJD infectivity and the total quantity of intravenous vCJD infectivity (i.v. ID_{50}) per plasma pool

Potential exposure to vCJD infectivity via UK manufactured FXI is a function of the probability that a plasma pool contains a donation with vCJD agent and the quantity of agent present in the donation and the larger assembled plasma pool. Prevalence of vCJD in the UK population directly influences the probability that a donation with vCJD agent will be present in a plasma pool.

III. A.1. Estimation of UK vCJD prevalence via two methods

The potential prevalence of vCJD in the UK was and continues to be dynamic and changes over time as people are exposed to the BSE agent, potentially infected with vCJD, develop the disease and eventually die. Variant CJD exposure and infections in the UK population likely occurred in proportion to the UK BSE epidemic which peaked in 1992. The first human vCJD cases were referred to UK public health authorities in 1994. To date, the number of cases in the UK reached a maximum of 28 in the year 2000, and since then has been declining annually with a total of 5 deaths in 2005. During the manufacture of FXI in the UK from plasma collected as late as 1997, the prevalence of human vCJD was likely higher than it is today in 2006 since exposure to BSE agent has ebbed and deaths of infected individuals lower the total number of infected persons. As of August, 2006 the UK recorded a total of 162 cases of definite or probable vCJD (CJD Surveillance Unit, 2006). However, the disease is likely to still be present in at least some members of the population as pre-clinical and asymptomatic infections. Most known infections and deaths have occurred in individuals who are homozygous methionine, or MM, at codon 129 of the PrP gene product. Prion positive tissue has been observed in individuals who are heterozygous methionine and valine (or MV) or homozygous valine (VV) at codon 129 (also called non-MM individuals) but to date none have developed symptoms and died of vCJD. Whether non-MM individuals will become symptomatic with vCJD or are capable of transmitting the disease is unknown.

In the scientific literature estimates of the rate of incubating vCJD cases in the UK have been derived from two potential sources: (1) epidemiological modeling studies based on the actual number of reported vCJD cases, and (2) a single study of surveillance testing for possible vCJD related protein accumulation, in tissues such as tonsil and appendix, which may or may not indicate a vCJD infectivity risk. This risk assessment used prevalence estimates derived both from the results of the appendix surveillance study, which yielded a vCJD prevalence estimate that may be representative of a possible higher prevalence scenario for potential vCJD infection, and from the epidemiological model, which yielded a lower vCJD prevalence estimate of potential vCJD cases based on a number of assumptions.

These estimates were used independently to generate two distinct estimates for UK vCJD prevalence for use in the model. Prevalence estimates derived from each data source are dramatically different. The mean vCJD case prevalence estimated using an epidemiological approach based on actual cases and projected case numbers, 4 infections per million people, is approximately 60 times lower than the mean infection prevalence estimated based on the study of tissue surveillance, 1 in 4,225. The data used to generate each prevalence estimate have limitations and uncertainties that contribute to the pronounced difference and uncertainty between the prevalence estimates, as noted above and cited below.

Two versions of the model were separately run using the two different estimates. Other parameters remained identical for each version. Results for each of the two different prevalence estimates were generated and are shown in the results tables for the model.

III. A.1.a. $P_{vCJD\text{-}Epi}$ - *Probability of vCJD-infected individual in UK population who will develop symptoms – determined by epidemiologic modeling-based prevalence estimate.*

$$P_{vCJD\text{-}Epi} = N_{vCJD\text{-}CE} / N_{pop\text{-}UK1997}$$

The probability of a vCJD-infected individual in UK population who will eventually develop symptoms is represented by the variable, $P_{vCJD\text{-}Epi}$. *It is derived by calculating the predicted number of vCJD cases that will progress to symptoms and be reported as a vCJD case to public health authorities divided by the size of the total population*, $N_{pop\text{-}UK1997}$, *in the UK in the year 1997.*

$N_{pop\text{-}UK1997}$ - this represents the estimated size of the UK population in 1997 which was 58 million (United Kingdom Office for National Statistics, 1997).

III. A.1.a.i. $N_{vCJD\text{-}CE}$ - *Estimated Number of vCJD-infected individuals in UK population using recorded vCJD cases (1997 – 2004) and epidemiological modeling based prevalence estimate*

Estimation of the UK vCJD prevalence during the period of interest during the manufacture of FXI from 1989 – 1997 was calculated by combining the number of reported vCJD cases during the time period from 1997 through 2004 with the future predicted cases of vCJD estimated by Clarke and Ghani (2005) for the period from 2005 to 2080. Although the total period spans from 1997 – 2080 (more than 80 years) and some of the future cases may not have been infected until after 1997, the calculations provide a conservative estimate that may slightly overestimate the number of cases as predicted in current epidemiological models. According to the United Kingdom National CJD Surveillance Unit (2006) (Table 2 below), there were 138 diagnosed vCJD cases through 2004. The variable, $N_{vCJD\text{-}CE}$, is the sum of 138 diagnosed vCJD cases, $N_{vCJD\text{-}Case}$, and the cases estimated by epidemiological modeling, $N_{vCJD\text{-}Epi}$, or an estimated 70 future cases. The sum of the expression is a total mean of 208 cases vCJD (95% CI: 148 – 328) and represented by the equation:

$$N_{vCJD\text{-}CE} = N_{vCJD\text{-}Case} + N_{vCJD\text{-}Epi}$$

III. A.1.a.ii. $N_{vCJD\text{-}Case}$ - *Number of reported vCJD cases in UK population 1997(and before) – 2004.*

Table 2. Number of Diagnosed vCJD Cases in the UK (Health Protection Agency, 2006)

Year	1997 and prior years	1998	1999	2000	2001	2002	2003	2004	Total
Diagnosed vCJD cases	12	17	17	28	25	16	16	8	138

III. A.1.a.iii. $N_{vCJD\text{-}Epi}$ - *Number of future vCJD-infected individuals in the UK population based on epidemiological modeling prevalence estimate*

Epidemiological modeling based prevalence estimates. Estimations of the future number of possible vCJD cases have narrowed considerably in the last decade and many recent estimates now predict a similar range for the number of possible future cases. Below (Table 3) are representative references of predicted future cases of vCJD that can be used to estimate the prevalence of vCJD in the UK. Our model uses the Clarke and Ghani (2005) estimate of 70 future cases of vCJD with a 95% confidence interval of 10 – 190 cases for the years 2004 – 2080. 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 vCJD infections per million((138+70) cases / 58 million) population.

There are some limitations associated with estimates of future vCJD cases and vCJD incidence in the UK generated by epidemiological modeling based on the current reported vCJD cases. 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 (e.g. less than 40 years, etc.). Fourth, many of the modeling approaches incorporate age-specific dependencies that influence exposure, susceptibility to the disease, and incubation period. Depending on the assumptions used, estimates of future cases of vCJD have varied considerably. Past estimates of vCJD cases from epidemiological models predicted from 250 to 440 future cases under certain assumptions (d'Aignaux *et al* 2001). As actual reported vCJD cases peaked in 2000 and have since been declining, predicted estimates of future cases have decreased (Boelle *et al* 2003, Clarke and Ghani 2005, Cooper and Bird, 2003).

There are additional uncertainties in predicting future vCJD cases that might arise from individuals with different genetic backgrounds and susceptibilities in the UK population. To date, all known cases of vCJD have occurred in individuals that were methionine homozygous (MM genotype) at the PRNP codon 129. Recent research has identified two individuals who were valine

homozygous (VV genotype, also called non-MM genotype) at PRNP codon 129 (Ironside *et al* 2006) among the three prion protein positive samples identified by Hilton *et al* (2004). Clarke and Ghani (2005) did incorporate the possibility of wider genetic susceptibilities in some of their estimates of future vCJD cases. However, because no cases of clinical vCJD have been identified yet in individuals with a non-MM genotype, it is uncertain whether such individuals will in fact develop clinical disease or be capable of transmitting the 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.

Table 3. Recent Representative Epidemiological Model-based Estimates of vCJD in the UK.

Reference	Years	vCJD cases (95% CI)	Comments
Clark and Ghani (2005)*	2004 - 2080	70 (10 – 190) (additional future cases)	Considers: Only MM cases (non-MM susceptibility*)
Cooper and Bird (2003)	2001 – 2005 2006 - 2010	164 (127 – 219) 88 (62 – 121) (additional future cases)	Considers: Only MM cases

* Clarke and Ghani 2005 provide estimates for two scenarios of wider susceptibility (in a non-MM population) using non-constrained assumptions but those estimates were not used in our model.

Assumption used in the model: The variable, $N_{vCJD\text{-}CE}$, is the sum of 138 vCJD cases diagnosed between 1997 and 2004, $N_{vCJD\text{-}Case}$, and the 70 future cases since 2004 estimated by epidemiological modeling, $N_{vCJD\text{-}Epi}$; the sum of the expression is a total mean of 208 cases vCJD (95% CI: 148 – 328) assuming the population of the UK in 1997 is approximately 58 million the prevalence of vCJD would be a mean of approximately 4 cases per million (208 cases / 58 million) population.

III. A.1.b. $P_{vCJD\text{-}Surv}$ - *Probability of vCJD-infected individual in UK population using the surveillance prevalence estimate*

Surveillance prevalence estimate. In vCJD patients the distribution of infectivity in tissues throughout the body is different than for other forms of CJD. Infectivity has been observed in the tonsil and spleen (Bruce *et al* 2001) as well as in the lymph nodes (Wadsworth *et al* 2001) of vCJD patients at the time of death. PrP^{TSE} has also been observed in an appendix sample of one asymptomatic vCJD individual several months prior to the onset of symptoms (Hilton *et al* 1998) and in the appendix sample of a symptomatic individual (Joiner *et al* 2004). Results from an ongoing surveillance study have been published in the literature on three separate occasions (Ironside *et al* 2000, Hilton *et al* 2003, 2004). The most recent report by Hilton *et al* (2004)

summarizes the results from the surveillance study, which included a total of 12,674 patients (results summarized in Table 4). Appendectomy samples from three of these 12,674 patients showed accumulation of prion protein. No tonsil biopsies showed such accumulation. Limitations to this study that contribute to uncertainty include the fact that this study was not controlled using a non-BSE exposed population and that a false positive interpretation could not be excluded. It is also not known whether this look at prions in tissues is a reliable marker for vCJD pre-clinical infection or for an individual's capability to transmit the infection through blood donation.

For the risk assessment model we assumed the sensitivity and specificity of the testing method for the accumulation of prion protein in tissues was 100%. The results of 3 positive samples in a total of 12,674 tissues tested (Hilton *et al* 2004) equates to an average rate of vCJD in the UK population of 1 in 4,225 or a mean of 237 infections per million (95% CI: 49 – 692 cases per million) for all age groups in the study. The authors (Hilton *et al* 2004) indicated that approximately 60% of the samples tested (from approximately 7,600 patients) came from patients 20-29 years of age. Furthermore, demographic information of reported vCJD cases (Table 5) indicated that the younger population that was deliberately oversampled in this study may have been more susceptible to the disease. The vCJD prevalence among UK donors might, therefore, be over-represented by the prevalence of 20-29 years age group derived from the surveillance study. To account for the age-specific bias in the sampling of tissues we calculated the age-specific prevalence for the 20-29 year old group and used that information to estimate the age-specific UK vCJD prevalences for the other age groups. To account for the age of individuals and populations in the model we assumed that there were 3 positive samples among the 7,600 20-29 year old patients in the Hilton *et al* study (2004). This yields an approximate UK vCJD prevalence of 1 infection in 2,500 individuals aged 20-29 years. This estimate is equal to approximately 400 infections per million for which we assumed a 95% CI of 100-1200 cases per million. We then derived the prevalences for the remainder of the UK donor population by determining the proportional difference between the vCJD prevalence from the tissue study group and the number of actual reported vCJD cases for donors in the 20-29 years age group. This proportion was then applied to the remaining age groups in the distribution of reported vCJD cases to determine the prevalence for each age group. By multiplying our extrapolated vCJD prevalence for incubating cases times the total donor population we were able to estimate the number of possible incubating vCJD cases in each UK donor age group. We assumed that a plasma pool used to manufacture Factor XI product in the UK in the 1990s consisted of 20,000 donations, and all donations came from different donors. The estimated prevalence was then used to generate variables and parameters representing the potential number of vCJD donors or donations that might be present in a plasma pool.

However, 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 abnormal prion protein in three of 12,674 adequate appendix samples studied. First, because the stage of vCJD infection during which the appendix first accumulates detectable amounts of abnormal prion protein is not known and because the accumulations might not be uniformly distributed throughout the tissue, the prevalence of infection might have been underestimated. Second, because the study design (lacking examination of a large number of similar appendices from a non-BSE-epidemic country) did not permit an estimate of specificity of the method or an independent confirmation of results, it is possible that the results might have been false positives

leading to an overestimation of prevalence. In their paper the authors stated: "Although immunohistochemical accumulation of PrP in lymphoreticular tissues has not been demonstrated in any disease other than vCJD, the significance of the positive samples in this study is not certain. In one case, the immunohistochemical pattern of immunoreactivity resembled that seen in appendix tissue from pre-clinical and autopsied cases of vCJD, but in the other two cases, a more finely granular pattern of staining was present in relation to follicular dendritic cells, raising the possibility that these may be false positives. However, we have been unable to demonstrate PrP immunoreactivity in a range of other disorders including other human prion diseases, neoplastic disease, or a range of inflammatory conditions."

Assumption used in the model: The higher prevalence estimate of vCJD infection in the UK population was based on surveillance studies of tonsils and appendices (Hilton *et al* 2004) and assumed to be a mean of 1 in 4,225 (1/20,300 to 1/1,450 - 95% CI).

Table 4. Summary of Surveillance Testing of Tissues including Tonsil and Appendix in the UK.

Reference	Ages of population examined	Years tissue taken	Number of positives	Total samples examined	Rate per million (95% CI)
Hilton *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)

Table 5. Reported vCJD Cases (Hilton *et al* 2004).

Age group	<10	10-14	15-19	20-24	25-29	30-34	35-39	40-44	45--49	50-54	55-59	60-64	65-69	>70
Reported vCJD cases (through 2003)	0	5	27	32	30	22	13	5	3	5	0	5		

III. A.2. Estimation of probability that infectivity will be present in blood (prionemia) in vCJD infected individuals at time of donation

Animal studies suggest that TSE infectivity is likely not present in the blood during the early stages of infection but rather manifests in blood during the later stages of the disease [reviewed in Brown (2005)]. Humans infected with vCJD are assumed in the model to display a similar pattern of infectivity in the blood (here termed "prionemia"). Accordingly, the model assumes that infectivity is present in the blood or plasma of donations from vCJD infected donors during the last

half of the incubation period. This means donations of blood or plasma from these individuals, especially in the early stages of the disease, may not contain vCJD agent and thus, at times, may not be capable of transmitting infection to recipients of whole blood, plasma, or plasma derivatives.

Calculation of the occurrence of infectious agent in blood of infected donors requires specific information or assumptions about the time or year of exposure to BSE and subsequent vCJD infection, and incubation time of the disease to determine the timing and occurrence of the last half of the incubation period, when infectivity is assumed to be most likely present in the blood of a vCJD infected donor. Because FXI was manufactured in the UK from the plasma of UK donors it was assumed that dietary exposure to the BSE agent led to subsequent vCJD infection. It was further assumed that the number of human infections in a specific year from 1980 to 1996 was proportional to the magnitude of the BSE epidemic in the specific year. Food chain control was in place in the UK in 1996. Therefore, we assume that the risk of exposure to BSE agent through dietary exposure after 1996 in the UK was negligible. Since the UK product, used under IND in the US, was manufactured from 1989 through 1998 – the model does not consider possible human exposure to the BSE agent after 1997. It was assumed that there is a lag of 6 months to one year from the time plasma is collected until it was manufactured/distributed as final product; therefore, plasma collected in 1997 was assumed manufactured into product in 1998.

III. A.2. a. BSE_y-*BSE cases reported in year y*

To estimate the relative magnitude of possible human exposure to the BSE agent in the UK, data on number of cases of BSE reported in cattle in the UK from prior to 1987 through 1996 were used as a relative metric of annual human exposure to BSE infectivity. We assumed that the amount of BSE agent that entered the UK food supply after 1996 was negligible. The number of human vCJD infections in any given year was assumed to be proportional to the number of BSE cases in cattle in a given year compared to the total number of cases that occurred through 1996. For example, out of a total of 169,473 BSE cases observed (data shown in Table 6) through1996, 37,280 cases or 22% of all cases (37,280/169,473 = 0.22) were observed in 1992. Proportionally, it would be predicted that approximately 22% of all human vCJD infections that occurred through 1996, would have occurred in 1992.

Data used in the model: Data from the World Organization for Animal Health (OIE, 2006), shown in Table 6, was used to determine the number of cases of BSE reported in the UK

Table 6. Number of BSE Cases (Cattle) Reported in the UK from 1980 through 1996 (OIE, 2006).

Year	1987 and before	1988	1989	1990	1991	1992	1993	1994	1995	1996	TOTAL
Number cases of BSE reported in the UK	446	2514	7228	14407	25359	37280	35090	24438	14562	8149	169,473

Assumption used in the model: There is a six month to one year (or longer) lag from the time plasma was collected from UK donors until it was manufactured into FXI for distribution. For

example, the model assumes that FXI distributed in 1998 was manufactured from UK plasma collected in 1997 or earlier.

III. A.2. b. $P_{infect-y}$-*Probability an infection occurring in year y - calculated by the equation:*

$$P_{infect-y} = BSE_y / \sum_{y=1980}^{1996} BSE_y$$

That is, the probability of infection with vCJD in a given year ($P_{infect-y}$) is a proportion equal to the number of reported BSE cases in the given year divided by the total reported number of BSE cases from 1980 through 1996. The summation in this section covers years 1980 through 1996 and does not address human exposure to the BSE agent in the UK after 1996 because the human exposure risk to the BSE agent thereafter was assumed to have dropped precipitously. After the announcement of 10 human vCJD cases in the spring of 1996, the UK implemented strict measures to prevent BSE infected cattle from entering the human food supply. Although cases of BSE were reported in the UK after the measures were imposed in 1996, the likelihood that BSE containing tissue entered the human food supply was low. Therefore, the model assumes that food-borne exposure to the BSE agent in the UK after 1996 was negligible.

Assumption used in the model: The probability of a vCJD infection occurring in a specific year is a function of exposure in that specific year, which is proportional to the number of BSE cases reported in that specific year (more BSE cases higher probability of getting infected) compared to the total BSE cases for all years through 1996.

III: A.2.c. P_{LH-y} – *Probability that the blood of an individual infected in year y will contain vCJD agent in the year 1997*

The models considered the possibility that although patients may be infected with vCJD, infectivity may not actually be present in the blood or plasma at the time the donation is collected. This phenomenon lowers the apparent prevalence of prionemia in infected donors meaning that many of the donations from infected individuals will not contain vCJD agent and would presumably pose little risk of transmitting vCJD. As discussed earlier, animal studies demonstrate that TSE agents appear in the blood during later stages of the disease. Accordingly, the FDA model assumes that vCJD agent was present in the blood of vCJD infected individuals during the last half of the incubation period and products derived from donations with infectivity may lead to exposure to vCJD (see below).

Assumption used in the model: FXI was made from UK donor plasma collected through 1997. For modeling purposes, we made a conservative assumption that the vCJD risk for all plasma collected for the manufacture of FXI used in the US was equal to the vCJD risk for plasma collected in the year 1997. We assumed that the vCJD risk for 1997 was likely the highest among all years that UK plasma was collected and used to make FXI used in the US because the total number of vCJD infections in the UK population (and the vCJD prevalence) were likely at or near their peak. Presumably vCJD prevalence would begin to decrease in 1997 and in subsequent years as the number of new vCJD infections declined due to the implementation of the stringent UK food chain controls of 1996. In addition to the decline in new vCJD infections, the vCJD prevalence and risk for the UK population likely started to decrease around 1997 and in subsequent years as patients died from the disease.

Assumption used in the model: The variability and uncertainty of the incubation period of vCJD (IP_{vCJD}) is represented mathematically by a gamma distribution, specifically Gamma (4.7, 3.6). A gamma distribution is usually used to represent intervals between events, in this case, the time from infection to the emergence of symptomatic disease. The distribution is defined by two parameters, one that produces the shape of the curve and a second that generates the scale between events which for vCJD is the mean incubation period of 14 years. Similar statistical methods and estimates for the vCJD incubation time have been used by others (Ghani *et al* 2004, Clarke and Ghani 2005).

IP_{vCJD} = The incubation period of vCJD was calculated in the model using a gamma distribution represented by the expression Gamma (4.7, 3.6) and expressed as:

IP_{vCJD} = Gamma (4.7, 3.6)

Assumption used in the model: The vCJD agent is present in the blood of infected individuals only during the last half of the incubation period. This assumption was expressed mathematically in the model as the probability that the incubation period of the disease was less than or equal to twice the elapsed period (1997-y). The period (1997 – y) is the number of elapsed years from the time of initial infection of an individual (in year *y*) until plasma was collected in the year 1997. For example, if an individual was infected in year y (for instance in 1987), and their blood or plasma was collected in 1997, the time since infection would have been (1997-y) years (1997 – 1987 or 10 years). In our example if we assume that the vCJD incubation period is 15 years, which is less than twice the elapsed period of 20 years (i.e., two times 10 years) the model would predict that the donor had vCJD agent in their blood in 1997.

The probability that an individual had the vCJD agent in their blood in year 1997 was represented in the model using the expression:

$P_{LH\text{-}y}$ = Cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)

III. A.2. d. P_{LH}- *Probability of an infected individual having vCJD agent present in their blood (prionemic) in year 1997.*

The probability that an individual infected in year *y* had the vCJD agent in their blood in the year 1997 was estimated in the model by determining if the incubation period of the disease was equal to or shorter than twice the elapsed period (1997-y). The period (1997 – y) is the number of elapsed years from the time of initial infection of an individual (in year *y*) until plasma was collected in the year 1997. Overall the probability that an individual was infected during the whole period between 1980 and 1996 is the sum of the product of $P_{infect\text{-}y}$ and $P_{LH\text{-}y}$, for y equal to each year from 1980 through 1996. An individual potentially could be exposed to the BSE agent and acquire vCJD infection in any year between 1980 and 1997. Therefore, the probability that an individual was infected during the period 1980-1997 and was prionemic in year 1997 was calculated by the equation:

$$P_{LH} = \sum_{y=1980}^{1996} (P_{infect-y} \times P_{LH-y})$$

III. A.2. e. $P_{vCJD-LH}$-The prevalence of prionemia among the UK donors in year 1997 is represented by the equation:

$$P_{vCJD-LH} = P_{vCJD} \times P_{LH}$$

The prevalence of prionemia among the UK population for the year 1997, $P_{vCJD-LH}$, shown in the equation above is a product of the probability a person will have vCJD (P_{vCJD}) times the probability they were prionemic, P_{LH-y}. The probability of vCJD occurring in the UK population was estimated for two distinctly different vCJD prevalences as described previously in section III. A. 1. The first prevalence estimate of 1 in 4,225 was based on an surveillance study of lymphoreticular tissues conducted in the early to mid-1990s in the UK (Hilton *et al* 2004) and may represent potential vCJD infections. The second prevalence estimate of 4 vCJD infections per million was based on epidemiological modeling of reported UK vCJD cases and is more reflective of known and potential vCJD cases (Clarke and Ghani 2005). However, given the uncertainty and disparity between each of these prevalence estimates it is difficult to say with any precision which, if either, is the best estimate for the potential infectivity of donors.

III. A. 3. Estimation of probabilities that a plasma pool contains a vCJD donation and probable number of vCJD donations per plasma pool

Estimation of the probability and number of vCJD donation(s) in a plasma pool in the model is a function of two factors:

- The prevalence of prionemia among the UK donors
- Number of donors per pool

Two versions of the model were used to generate two separate sets of results: one version of the model used the higher vCJD prevalence estimate and a second version of the model used the lower vCJD prevalence estimate; the prevalence estimates are described above. Results from each of the two versions of the model are shown in the risk characterization section (Section IV) of this document.

III. A.3. a. D_{Tpool} - *Total number of donors per pool*

Assumption used in the model: FXI was manufactured from a pool of approximately 20,000 plasma donations. Each donation was presumed to come from different donors. Therefore,

$D_{Tpool} = 20,000$ *donors*

III. A.3. b. D_{vCJD} -*Probable number of vCJD donors or donations present per plasma pool*

Assumption used in the model: The number of vCJD donors per plasma pool is represented by a binomial distribution defined by two arguments alpha (α) and beta (β) (represented in the model by the expression Riskbinomial (α, β)). Alpha represents the total number of donors per plasma pool (D_{Tpool}), which is 20,000 in this case. Beta is the probability of a donor to have prionemia when donating, which is the prevalence of prionemia among the UK population in year 1997 ($P_{vCJD\text{-}LH}$ calculated in III.A.2.e). D_{vCJD} is represented by the expression:

D_{vCJD} = Riskbinomial (α, β) = Riskbinomial (D_{Tpool}, $P_{vCJD\text{-}LH}$,) or Riskbinomial (20000, $P_{vCJD\text{-}LH}$)

The probable number of vCJD donors (donations) present in a single plasma pool was estimated for the two UK vCJD prevalences discussed in section III. A. 1 (based on the tissue surveillance study and epidemiological modeling-based methods).

III. A.3. c. $P_{vCJD\text{-}pool}$-*Probability of a plasma pool containing a vCJD donor (donation)*

The probability of a plasma pool containing a vCJD donor (donation) depends on number of donors who contribute to a pool and vCJD prevalence among the UK donor population.

Assumption used in the model: The number of vCJD donors per plasma pool is represented by a binomial distribution defined by two arguments alpha (α) and beta (β) (represented in the model by the expression Riskbinomial (α, β)). Alpha represents the total number of donors per plasma pool (D_{Tpool}), which is 20,000 in this case. Beta is the probability of a donor to have prionemia when donating, which is the prevalence of prionemia among the UK population in year 1997 ($P_{vCJD\text{-}LH}$ calculated in III.A.2.e). Cumulative frequency of binomial distribution (D_{Tpool}, $P_{vCJD\text{-}LH}$) at X=0 represents the probability of a plasma pool not to contain any infected donor (donation); therefore, the probability a plasma pool containing a vCJD donor (donation) was calculated by:

$P_{vCJD\text{-}pool}$= 1- Cumulative frequency of Binomial(D_{Tpool}, $P_{vCJD\text{-}LH}$), at x=0

III. A.4. Estimation of Quantity of vCJD agent per donation and in plasma pools used in manufacturing UK FXI

III. A.4.a. I_D - *Estimated Total Infectivity (or i.c.ID_{50}) per vCJD donation*

The model estimates the total infectivity or i.c. ID_{50} per vCJD donation as a function of the volume of plasma per donation multiplied by the infectivity associated with plasma. The i.c. ID_{50} in plasma is calculated from the percentage of infectivity that is estimated to be present in plasma. The model expresses intracerebral (i.c.) vCJD infectivity in terms of the i.c. ID_{50} as the amount of tissue material, in this case blood or plasma, that when injected into the brain causes infection in 50% of the population. More details on the variables and parameters for this portion of the model are described below.

III. A.4.a. i. D_V - *Amount of recovered plasma per donation*

D_V - *The amount of plasma recovered from a unit of whole blood is represented in the model by a single value point estimate of 200 milliliters*

A unit of whole blood has a volume of approximately 450 milliliters. The plasma portion is separated from the cellular portion of a unit of whole blood within hours of its collection.

Assumption used in the model: The model assumes that approximately 200 milliliters (mls) of plasma can be separated away from the blood cells.

III. A.4.a. ii. I_{bl} - *Infectivity of vCJD (or i.c.ID_{50}) present in infected blood per ml*

I_{bl} - *The potential amount of vCJD agent present in whole blood collected from vCJD infected individuals is represented in the model by a log normal statistical distribution of (2, 12, 30)* i.c. ID_{50}/ml *(5^{th} percentile, most likely, and 95^{th} percentile) with minimum and maximum of 0.1 and 1,000, respectively.*

Based on limited available data (see below), FDA believes that the quantity of infectivity present in blood from a vCJD infected individual in i.v. ID_{50} is likely represented by a distribution with the following characteristics: Minimum value = 0.1, 5^{th} percentile = 2, Most likely value = 10, 95^{th} percentile = 30, and Maximum value= 1,000 i.v. ID_{50}. Given the possible parameters, statistical distributions were fitted to the selected parameters using Best Fit part of the @Risk Professional software package (Palisade Corporation, New York). Using the software we determined that a log normal statistical distribution (of (2, 12, 30) i.c. ID_{50}/ml (5^{th} percentile, most likely, and 95^{th} percentile) with minimum and maximum of 0.1 and 1,000, respectively) provided the best fit.

Conclusions from several research groups arrive at somewhat similar estimates for the quantity of infectivity that might be present in the whole blood of mice and hamsters. Using a mouse model and human CJD Brown *et al* (1999) found a range from 0.5 to 15 mouse i.c. infectious units (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). One IU 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. ID50 with the possibility that at times the infectivity present in blood may exceed this range.

Assumption used in the model: Whole blood collected from a vCJD-infected individual can vary from person to person in the quantity of infectivity it contains. The model used a log normal statistical distribution to represent the variability and uncertainty of the quantity of infectivity in

blood. It was assumed that whole blood from an infected person potentially carries a minimum of 0.1 i.c. ID_{50} per ml, a 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).

III. A.4.a. iii. $I_{Pl\text{-}perc}$ - *Percentage infectivity associated with plasma (i.c. ID_{50}/ml)*

$I_{Pl\text{-}perc}$ - *The percentage of vCJD agent associated with the plasma portion of whole blood is represented in the model by a single value point estimate of 58%.*

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.

III. A.4.a. iv. I_D - *Total infectivity (or i.c.ID_{50}) per vCJD recovered plasma donation*

Total i.c.ID_{50} per vCJD donation is represented by the equation:

$$\mathbf{I_D = D_V \times I_{bl} \times I_{Pl\text{-}perc}}$$

In this case I_D or total infectivity or i.c. ID_{50} per vCJD donation equal to the volume of plasma per donation (D_V) multiplied by the infectivity associated with plasma which is derived from the ID_{50}s present in blood (I_{bl}) times the percentage of infectivity present in plasma ($I_{Pl\text{-}perc}$). Total vCJD infectivity is expressed in terms of the ID_{50} or the infectious dose needed to cause infection in 50% of the population.

Assumption used in the model: One ID_{50} is the amount of material containing infectious agent that has a 50% probability of causing infection in an individual or population.

III. A.4.a. v. $A_{ic\text{-}iv}$ - *Adjustment for intravenous route of infection*

$A_{ic\text{-}iv}$ - *is represented in the model by a uniform distribution between 1 and 10. This variable provides an adjustment for the difference in efficiency between the intravenous and intracerebral routes of introduction in initiating infection.*

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) (Oct 31, 2005) the range of efficiency of i.v. route (versus the i.c. route) was assumed in the model to range between the values of 1 and 10.

Assumption used in the model: Exposure to infectivity by the i.v. route is between 1 and 10 times less efficient at causing infection than introduction via the intracerebral route. Using a value of 1 for the ratio of the lower bound of the efficiency is a conservative estimate and assumes that theoretically there would be no difference between the efficiency in initiating infection between the i.c. and i.v. routes.

III. A.4.b. $\mathbf{I_{iv\text{-}pool}}$ - *Total intravenous infectivity or i.v.ID_{50} per plasma pool of 20,000 donors*

The output of this component of the model, total i.v. ID_{50} per plasma pool, is represented by the equation:

$$\mathbf{I_{iv\text{-}pool}} = \frac{\mathbf{D_{vCJD}} \times \mathbf{I_D}}{\mathbf{A_{ic\text{-}iv}}}$$

Total intravenous vCJD infectivity per plasma pool ($\mathbf{I_{iv\text{-}pool}}$) was calculated in the model by multiplying the total vCJD donations per pool, $\mathbf{D_{vCJD}}$, by the total quantity of infectivity, $\mathbf{I_D}$, (ID_{50}) per donation and dividing the product by the adjustment for intravenous route of introduction, $\mathbf{A_{ic\text{-}iv}}$.

III.B. Total i.v. ID_{50} per vial after processing / production of FXI

This component of the model estimates the total i.v. ID_{50} of vCJD infectivity that may be present in a vial of FXI that was manufactured in the UK and used in the US under IND. Production of FXI in the UK involved the pooling of recovered plasma from a pool of approximately 20,000 donations. Some steps during production may be expected to remove vCJD infectivity, thereby reducing the amount in the finished product. There were two steps that reduced the amount of infectivity. First, the original starting plasma material was approximately 5,000 kg of plasma from which approximately 800 kg was removed and used to produce the FXI product. This means that only approximately 16% (800/5,000) of infectivity from the large pool of 20,000 donations remained. Finally, because of the types of processing steps used in the manufacture of FXI we assumed a most likely reduction in infectivity of 2 $\log_{10}$ (or 99%). These two steps would result in a significant reduction in the amount of vCJD present in the FXI product from the UK. However, the model assumes that infectivity would only be reduced and not eliminated. Therefore, if present in the original donations, some vCJD infectivity is predicted by the risk assessment model to persist, following manufacturing, in some FXI final product produced in the UK and may have then posed a risk of transmitting vCJD to patients that received the product.

III.B.1. $R_{W\%}$ - *Percentage of pool used to manufacture FXI*

The initial starting amount of material from 20,000 recovered plasma donations in the UK was estimated to weigh 5,000 kg of which 800 kg (or 16%) of the material was removed and used to produce FXI. As stated earlier this step represents an 84% reduction in the quantity of starting materials; consequently any infectivity that may be present would also be removed from the pool of 20,000 plasma donations.

Assumption used in the model: Approximately 16% of starting plasma material from 20,000 donations was used in the manufacture of FXI.

III.B.1.a. W_{st} - *Weight of starting product*

Assumption used in the model: Weight of starting product is represented in the model by a single value point estimate of 5,000 kg.

III.B.1.b. W_m - *800kg portion removed and used to extract FXI*

W_m - *Portion of total product used in manufacturing is represented in the model by a single value point estimate of 800 kg.*

Assumption used in the model: 800 kg of material was removed and used to produce FXI.

Portion used is represented by the equation and calculations:

$R_W = W_m / W_{st}$
$R_W = 800 / 5{,}000$
$R_W = 0.16$
$R_{W\%} = 16\%$

The removal of 800 kg or 16% of the pooled product from the original starting material of 5,000 kg represents an 84% reduction in the amount of i.v. ID_{50}s present in the original pool of 20,000 donations.

III.B.2. R_{Log} - *Log reduction in ID_{50} during processing*

Represented in the model by a triangular statistical distribution representing a reduction in ID_{50} during processing of (0, 2,4) Log_{10} i.v. ID_{50}/ml *(minimum, most likely, and maximum).*

TSE agents are highly resistant to conventional inactivation methods such as alcohol, other solvents, and heat denaturation. At least one step during the production of FXI has the potential to reduce the amount of vCJD agent present by physical separation (partitioning). Based on available

scientific data for similar processes, as well as studies of prior reduction during manufacturing of different plasma products, CBER has estimated by internal expert opinion that the level of removal of the vCJD agent during processing corresponds to a reduction of a minimum of 0, a most likely reduction of 2 Log_{10} ID_{50}, and a maximum possible reduction of 4 Log_{10} ID_{50} per ml. Empirical verification of these estimated levels of reduction has not been done to our knowledge.

Assumption used in the model: Processing reduction is represented by a triangular statistical distribution representing a reduction in ID_{50} during processing of (0, 2, 4) Log_{10} i.v. ID_{50}/ml (minimum, most likely, and maximum).

Assumption used in the model: The model assumes that infectivity is reduced but not entirely eliminated from plasma and the product during processing. Therefore, although the amount of *ID_{50}* vCJD agent may be reduced, the percentage of pools and vials containing the vCJD agent still remains the same.

III. B. 3. I_{pp} - *Total i.v. ID_{50} present per pool of FXI post-processing*

$$I_{pp} = I_{iv\text{-}pool} \times R_W \times 1/10^{RLog}$$

The total i.v. infectivity (i.v. ID_{50}s) present in processed product (I_{pp}) is a function of the total infectivity present in the pool ($I_{iv\text{-}pool}$) prior to processing steps that might reduce the amount of infectivity present in the final FXI product. The infectivity in the pool ($I_{iv\text{-}pool}$) is multiplied by R_W because only 800kg out of the original 5,000 kg (or 16%) of starting plasma pool is used and multiplied by processing reduction steps (R_{Log}), which are expected to reduce the infectivity in the final FXI product by a most likely of Log_{10} 2 (or 99%), or by a maximum level of Log_{10} 4 (or 99.99%).

III.B.4. Y_{fT} - *Total yield of FXI from plasma pool*

FXI is present in trace amounts in human plasma.

Assumption used in the model: The estimated yield of FXI per kg plasma was approximately 150 to 180 units, subsequently the model estimates the total yield of FXI as 120,000 to 144,000 units per batch of 800 kg starting material. FXI was distributed in vials containing 1,000 units each (BPL, 2001).

The yield of FXI from the starting material was represented in the model by the equation:

$$Y_{fT} = W_M \times Y_{f\text{-}kg}$$

III.B.4.a. $Y_{f\text{-}kg}$ - *Yield of FXI per kg of plasma*

Yield in the model was estimated to be between 150 to 180 units of FXI per kg plasma This variable was represented in the model using a uniform distribution with a minimum yield of 150 units and a maximum yield of 180 units per kg of starting plasma material.

III.B.5. V_u - *Vial size or number of units per vial*

It was assumed that each vial contained 1,000 units of FXI.

III.B.6. V_T - *Total number vials produced*

The FXI product was aliquoted into vials with approximately 1,000 units each, and the total number of vials produced was estimated in the model by the simple equation:

$$V_T = Y_{fT} \ / \ V_u$$

III.B.7. I_{vial} - *Total i.v. ID_{50} per vial*

The total i.v. ID_{50} present in each vial of FXI was estimated by dividing the total estimated i.v. ID_{50} per pool (I_{pp}) of starting material by the total number of vials produced. Calculations used in the model are represented by the equation:

$$I_{vial} = I_{pp} \ / \ V_T$$

or including all component variables by the equation:

$$I_{vial} = \left[\frac{D_{vCJD} \times D_V \times I_{bl} \times I_{pl}}{A_{ic\text{-}iv}} \right] \times R_W \times 1/10^{RLog} \Big/ \ (W_m \times Y_{f\text{-}kg} \ / \ V_u)$$

Summary of variable names used above are:
D_{vCJD} - *Total number of vCJD donations per pool*
D_V - *Amount of recovered plasma per donation*
I_{bl} - *Infectivity of vCJD (or i.c.ID_{50}) present in infected blood per ml*
I_{Pl} - *Proportion infectivity associated with plasma (i.c.ID_{50}/ml)*
$A_{ic\text{-}iv}$ - *Adjustment for intravenous route of infection*
R_W - *Portion of pool used to manufacture FXI*
R_{Log} - *Log reduction in ID_{50}s during processing*
W_m - *Portion of total product used in manufacturing(800 kg).*
$Y_{f\text{-}kg}$ - *Yield of FXI per kg of plasma*
V_u - *number FXI units per vial*

III.C. Utilization by patients with FXI deficiency undergoing surgery

FXI normally circulates in the human bloodstream at a concentration of approximately 50 u/dl (5ug/ml) and has been observed by some researchers to be present at concentrations as high as 70 u/dl. Those with very severe FXI deficiency have < 1 unit per deciliter (u/dl) of blood (BPL, 2001). The commonly used target treatment dose ranged from 20 – 50 u/ kg body weight. Individuals at risk for excessive bleeding prior to surgery can receive prophylactic treatment at the recommended dose in anticipation of surgery. Because the half-life of FXI is approximately 52 hrs (Mannucci *et al* 1994), patients may need additional post-surgical maintenance treatments every 2 to 3 days to maintain therapeutic levels.

III.C.1. Total Dose for Pre- and Post-surgical treatment with FXI

Published data are available on the per surgical event utilization of FXI (Mannucci *et al* 1994, Aledort *et al* 1997) manufactured in the UK so that potential exposure to the vCJD agent can be estimated more accurately. It is difficult to determine the exact dose given to each patient without the patient medical record because only the dose per body weight of 20 – 50 u/kg is provided. The scenarios described below approximate the amount of FXI given per patient to provide insight into the possible magnitude of risk. In this portion of the model we lay out three possible scenarios:

Scenario 1 – Treatment of a 60 kg individual with FXI (20 – 50 u/ kg) once during or after surgery for a total patient dose of approximately 3,000 units.

Scenario 2 - Treatment of a 60 kg individual both pre- and post-surgery with a total of approximately 9,000 units of FXI.

Scenario 3 - Treatment of a 60 kg individual both pre- and post- surgery with a total of approximately 15,000 units of FXI.

III.C.1.a. D_{Pre} - *Prior to major Surgery - doses of 20 – 50 u/ kg given*

Assumption used in the model: The dosage prior to surgery is approximately 20 – 50 u/kg body weight. This dosage scheme is represented in the model with a point estimate.

D_{Pre} = **Dose** (*20 – 50 u/kg*) x **Patient weight (kg) x Number treatments**

III.C.1.b. D_{Post} - *Post-surgical maintenance of 20 – 50 u/kg every 2 - 3 days*

Assumption used in the model: The post-surgery maintenance dosage is assumed to be *20* – 50 u/kg given every two to three days. This dosing scheme is represented in the model with a point estimate.

D_{Post} = **Dose** (*20 – 50 u/kg*) x **Patient weight (kg) x Number treatments**

III.C.1.c. D_T - Total FXI doses given per patient per surgical procedure

The output is a sum of all doses of FXI given pre- and post-surgery to prevent or minimize bleeding by FXI deficient patients. The sum of doses is represented by the equation:

$$D_{Tu} = D_{Pre} + D_{Post}$$

III.C.2. Scenario 1: Treatment 60 Kg individual with 3,000 units FXI

A 60 Kg person receives one dose FXI to minimize potential bleeding episodes at a concentration of *20* – 50 u/kg would receive a total of approximately 3,000 units. Output is the estimated total units FXI received and estimated vCJD ID_{50} received. At this time, the actual dosing that patients received is not known.

III.C.3. Scenario 2: Treatment with 9,000 units FXI

Assumption used in the model: During preparation and recovery from surgery the model assumes that a patient receives a total dose of 9,000 units FXI to minimize potential bleeding episodes. Output is the estimated total units FXI received and estimated vCJD ID_{50} received.

Scenario 2 is similar to amounts of FXI given in three dosing regimens given at 20 – 50 units per kg body weight -one treatment given prior to surgery and two treatments given during post-operative recovery (Mannucci *et al* 1994).

III.C.4. Scenario 3: Treatment with 15,000 units FXI

Assumption used in the model: During preparation and recovery from surgery the model assumes that a patient receives a total dose of 15,000 units FXI to minimize potential bleeding episodes. This scenario may involve a 60 kg individual that receives approximately five treatments both prior to and following surgery at a dose of 20 – 50 u/kg.

Output Scenario 3: Estimated Total units FXI received and estimated vCJD ID_{50} received.

IV. RISK CHARACTERIZATION

The risk characterization section of the risk assessment integrates the hazard identification, hazard characterization and the exposure assessment components to arrive at estimates of the risks posed by a hazard.

In this risk assessment data for hazard characterization are lacking, so we could not develop a human vCJD dose-response. The dose-response relationship provides information needed to use the exposure (dose) assessment results to estimate the probability of adverse responses including infection, illness or mortality – based on assessment of exposure (dose) to the hazard. Many TSE models and risk assessments, including our model, use the ID_{50}, or amount of material that leads to infection in 50% of the population, as a semi-quantitative estimate of the amount of TSE agent. It is possible to interpret the ID_{50} as representing a linear dose-response relationship or linear relationship between exposure and the probability of infection. In such a case exposure to 1 ID_{50} would suggest a 50% probability of infection, exposure to 0.1 ID_{50} would suggest a 5% probability of infection, and so on.

In assuming a linear dose-response relationship we have chosen a conservative approach with respect to the risk that may be present at decreasing exposure levels below an ID_{50}. However, it is possible that exposure to less than 1 ID_{50} may not result in infection. Given the limited data available, any extrapolation or interpretation has limited utility in actually estimating clinical outcomes such as infection and illness. Therefore, any estimate of the risk based on estimates of exposure to the vCJD agent through use of FXI will be imprecise and extremely uncertain.

IV.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 UK-manufactured FXI. 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 are provided in Appendix A. Where data were not available, simplifying assumptions were used in the model and are detailed in the preceding documentation. Assumptions used in the model are presented in tabular form in Appendix B. The model was run using @Risk software package (Palisades Corp, NY) to conduct the Monte Carlo analysis. Simulations of 10,000 iterations were run.

The risk assessment uses Monte Carlo simulation to randomly draw values from probability input distributions (which are statistical representations of input data) once per iteration; thousands of iterations are used to generate the model outputs as risk estimates. This simulation method is often used in situations when a model is complex, non-linear, or involves several uncertain parameters. The output generated is usually an aggregate distribution whose shape can be summarized using measures of central tendency (mean, median, mode) or with boundaries such as the 95% confidence interval (CI), the 5^{th} and 95^{th} percentiles (representing the 90% CI) or the range, bounded by the minimum and maximum values generated as part of the output. The strength of Monte Carlo analysis is that it generates resulting risk estimates as statistical distributions which reflect the underlying uncertainty and variability of the original input data and parameters.

The model provided predictions of estimated exposure to the vCJD agent in the form of intravenous (i.v.) ID_{50} in patients treated with UK-manufactured FXI. **Because an accurate dose-response relationship (or hazard characterization) for vCJD exposure and the probability of human illness has not been developed it is not possible to predict with any accuracy the probability of vCJD infection and illness in an individual exposed to the agent.**

IV. B. Results from the Model

Results from the model in Table 7 show the estimates of potential probabilities that a plasma pool used to manufacture FXI from UK donor plasma may potentially contain a vCJD donation and predicts the number of possible vCJD donations per pool. Using the epidemiological case based prevalence estimate (4 infections per million population) the modeling estimates that a mean of 1.6% of pools may contain a vCJD agent. Using the higher tissue sample surveillance– based prevalence estimate (1 in 4,225) as a possible higher prevalence scenario the model estimates that an average of 50% of pools may possibly contain vCJD agent. A more detailed version of Table 7 is provided in Appendix C (Table C.I.) and in addition displays the median estimates of the potential probabilities of a vCJD donation and number of vCJD donations per plasma pool.

Table 7. Potential Probabilities and Number of vCJD donations per Plasma Pool

	MODEL OUTPUT USING LOWER PREVALENCE ESTIMATE *vCJD Case Prevalence from epidemiological modeling ~4 per million (Clark and Ghani, 2005)*		*MODEL OUTPUT USING HIGHER PREVALENCE ESTIMATE* *vCJD Infection estimate from tissue surveillance study 1 in 4,225 (Hilton et al 2004)*	
	Mean	5th- 95th percentiles[a]	Mean	5th- 95th percentiles[a]
Probability pool contains vCJD donation	**1.6%**	1.1% - 2.1%	**50%**	18% - 77%
Number vCJD donations per pool	**0.02**	0 – 0[b]	**0.75**	0 - 3

[a] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FXI recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FXI plasma pool would be rare and more than 90% of FXI product lots (of vials) would not be predicted to contain vCJD agent.

Table 8 displays results from the model of estimates of risk for 3 different treatment scenarios. FDA recently reviewed the original IND protocols and patient treatment regimens – the three scenarios reflect representative treatment scenarios and the range of FXI quantities used in the original IND studies. The model indicates risk ranges from a low in Scenario 1, at the lower prevalence estimate, with a mean exposure of 3.11×10^{-3} i.v. ID_{50} and a mean estimated per person

(per treatment course) risk of vCJD infection of 1 in 643. The higher end of the range of risk is illustrated in Scenario 3, using the higher estimate of prevalence, the model estimated a mean exposure of 0.59 i.v. ID_{50} and a mean estimated per person (per treatment course) risk of vCJD infection of 1 in 3.4.

Readers may notice that the results for "mean potential vCJD risk per person" generated by the model using the low vCJD case prevalence estimate have 5th and 95th percentile values of 0 and 0, respectively (Table 8). Because at low vCJD prevalence the model results indicate that the chance of an infected donor (with infectious vCJD agent in their blood at the time of donation) donating to a plasma pool would be an infrequent event. The zero values for the 5th and 95th percentiles indicate that at least 95 percent of the time the model predicted the risk of possible vCJD infection was zero for FXI recipients because the vCJD agent was not present in FXI product as administered during treatment. However, 1.6% of the time FXI lots may contain the vCJD agent and this results in an average per person exposure that is greater than zero as shown for the low vCJD case prevalence under the column "Mean vCJD i.v. ID_{50}" in Table 8. Although the model suggests that exposure of FXI recipients may have occurred, particularly when the higher estimate of prevalence based on tissue samples is used, the large variability and uncertainties in the data used in the model, and in assumptions used in the model itself do not allow us at this time to determine if exposure to the vCJD agent, in fact, did or did not occur in FXI recipients and it is not possible to estimate the precise magnitude of risk faced by recipients of UK-manufactured FXI product. Also, the possibility of vCJD exposure and infection does not necessarily mean that an individual will go on to develop symptoms of vCJD or vCJD disease. A more detailed version of Table 8 is available in Appendix D (Table D.I.), which also displays the median estimates of the potential probabilities of a vCJD donation and number of vCJD donations per plasma pool.

Table 8. Mean Potential Exposure and Mean Potential Risk per Person per FXI Treatment Scenario.

		MODEL OUTPUT USING LOWER PREVALENCE ESTIMATE *vCJD Case Prevalence from epidemiological modeling ~4 per million (Clark and Ghani, 2005)*		*MODEL OUTPUT USING HIGHER PREVALENCE ESTIMATE* *vCJD Infection estimate from tissue surveillance study 1 in 4,225 (Hilton et al 2004)*	
Scenario	**Quantity* Factor XI Utilized (u*)**	**Mean potential exposure to vCJD i.v. ID_{50}**** (5^{th} - 95^{th} perc)[a]	**Mean potential vCJD risk*** per person** (5^{th} - 95^{th} perc)[a]	**Mean potential exposure to vCJD i.v. ID_{50}**** (5^{th} - 95^{th} perc)[a]	**Mean potential vCJD risk*** per person** (5^{th} - 95^{th} perc)[a]
Scenario 1: Treatment 3,000 u	3,000 u	3.11×10^{-3} (0 - 0)[b]	1 in 643 (0 - 0)[b]	0.12 (0 – 0.57)	1 in 17 (0 – 1 in 3.5)
Scenario 2: Treatment 9,000 u	9,000 u	9.33×10^{-2} (0 - 0)[b]	1 in 214 (0 - 0)[b]	0.36 (0 – 1.70)	1 in 5.6 (0 – 1 in 1.2)
Scenario 3: Treatment 15,000 u	15,000 u	1.55×10^{-2} (0 - 0)[b]	1 in 130 (0 - 0)[b]	0.59 (0 – 2.86)	1 in 3.4 (0 – 1 in 1)

*u - represents units of Factor XI – and is equivalent to the term "unit" or "units" used in this document
** Mean vCJD i.v. ID_{50} (per treatment course) - the average predicted quantity of vCJD agent an individual in a specific treatment group is predicted to receive based on the model.
***Mean potential vCJD risk per person – the per person risk of potential vCJD infection based on animal model dose-response information. Mean potential vCJD risk per person = Total mean quantity i.v. ID_{50} (per treatment course/per person) x 0.5 (50 % chance infection - ID_{50})
[a] The 5^{th}- 95^{th} perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model are expected to fall within this defined interval at least 90% of the time.
[b] For a 5^{th} and 95^{th} percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FXI recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FXI plasma pool would be rare and more than 90% of FXI product vials would not be predicted to contain vCJD agent.

IV. C. Sensitivity Analysis

Sensitivity analysis is used to identify the input parameter or parameters that have the greatest impact on the risk estimates generated by the model. Our goal in doing the analysis was to identify the key input parameters that have the greatest influence on annual exposure to the vCJD agent. Generally, sensitivity analysis is conducted by varying the values of key input parameters about a range of values and then evaluating the effects on the final risk estimate. The model was examined and candidate variables for the sensitivity analysis were chosen from the model that exhibited the largest potential for variability and/or uncertainty and those values are listed in Table 9. We conducted a type of sensitivity analysis called importance analysis which evaluates the impact of a minimum and a maximum value on the risk estimate and ranks the factors in the model based on their importance (or influence) on the risk estimate. Our analysis used two values, one at the 5^{th}

percentile (or minimum) value and one at the 95th percentile (or maximum) value to provide a reasonable estimate of impact across the range tested. Results from the analysis are displayed as tornado graphs (Figures 2.A. and 2.B.), which graphically shows the relative influence of each input parameter evaluated on the final model estimates. For the FXI risk assessment the output being monitored in the sensitivity and importance analyses was the predicted annual exposure (I_{yr}) to vCJD agent, quantified in i.v. ID_{50} units, to recipients of FXI.

The sensitivity analysis was run separately each time using one of the two surveillance estimates. The first analyses used the higher vCJD Infection prevalence estimate of 1 in 4,225 (or 237 per million) derived from a tissue surveillance study (Hilton *et al* 2004) For the purposes of this analysis we first adjusted the prevalence for donor age and the presence of infectivity in the blood during the last half of the incubation period, which generated a range about the adjusted HIGHER vCJD Infection prevalence ($P_{vCJD\text{-}AdjSurv}$) based on the tissue surveillance study with a 5th percentile value of 3 per million and a 95th percentile value of 135 per million. The second set of analyses used the lower vCJD Case prevalence estimate of ~1.8 per million based on epidemiological modeling from actual vCJD occurrence conducted by Clarke and Ghani (2005). As for the first analyses, prevalence was adjusted by donor age and the presence of infectivity in the blood during the last half of the incubation period which generated a range about the adjusted LOWER vCJD Case prevalence ($P_{vCJD\text{-}AdjEpi}$) based on epidemiologic modeling with a 5th percentile value of 0.5 per million and a 95th percentile value of 1 per million.

Table 9. Input Variables included in Importance Analysis

Name of input variable	Description of variables	Range for importance analysis
$P_{vCJD\text{-}AdjSurv}$	Adjusted Tissue surveillance-based prevalence (HIGH prevalence estimate): vCJD Infection prevalence (at last half incubation period) in UK donor (cases/million)	5th perc: 3 95th perc: 135
$P_{vCJD\text{-}AdjEpi}$	Adjusted Epidemiological modeling based prevalence(LOW prevalence estimate): vCJD prevalence (at last half incubation period) in UK donor (cases/million)	5th perc: 0.5 95th perc: 1
$A_{ic\text{-}iv}$	Adjustment factor for i.v. infectivity vs i.c. infectivity	Minimum: 0.1 Maximum: 1
I_{bl}	i.c. infectivity of infected human blood	Minimum: 2 Maximum: 30
Y_{VIII}	FXI Yield (u/L plasma)	Minimum: 150 Maximum: 180
R_{Log}	Reduction of infectivity during manufacturing	Minimum: 0 Maximum: 4
D_T	Annual usage of FXI (u/year)	Minimum: 3,000 Maximum: 15,000

The analysis was performed for each variable by doing two sets of simulations, each with 5,000 iterations. For each set of simulations the value of one testing variable was set at the minimum or 5th percentile value for the input distribution and the simulation run; for the second run the variable was set at the maximum or 95th percentile value and the simulation run. The results of all simulations and the ranking of input parameters by their importance are graphically depicted using

a tornado plot or graph as shown in Figures 2.A. and 2.B. The tornado plot displays the correlations between key inputs in the model and the model output of exposure. A tornado plot prioritizes the various input factors with the most influential factors at the top and those that are least influential or those with negative influence on the risk are at the bottom of the plot.

Figure 2. A. FXI Importance Analysis Ranking Influential Factors for Predicted Annual vCJD Exposure (I_{yr}) Using an Adjusted Tissue Surveillance-Based (HIGH) Prevalence Estimate. Tornado plot showing impact of input variables on estimated per treatment course exposure of FXI recipients.

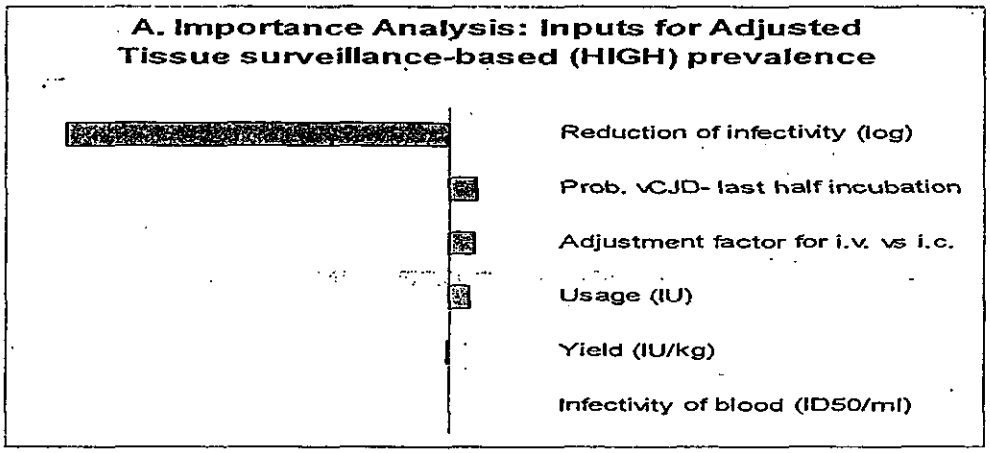
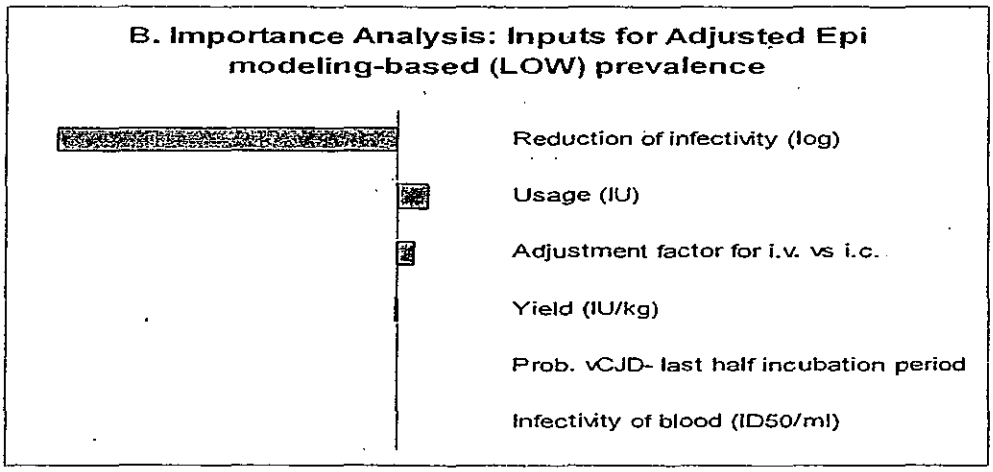


Figure 2. B. FXI Importance Analysis Ranking Influential Factors for Predicted Annual vCJD Exposure (I_{yr}) Using an Adjusted Epidemiological Modeling-Based (LOW) Prevalence Estimate. Tornado plot showing impact of input variables on estimated per treatment course exposure of FXI recipients.



The order of the influence of the specific input factors varies slightly when the importance analysis is conducted using the two difference prevalence estimates. The tornado plots in Figures 2 A and B both show that clearance or Log reduction of vCJD agent (R_{Log}) during the manufacturing

process is the dominant factor that influences the annual exposure or risk for a FXI recipient. The importance analysis suggests that changes in the input values for prevalence used in the analysis can cause some moderate yet visible changes in the rank order of the influence of the various input factors. For instance, using the HIGH prevalence estimate ranks the probability of vCJD agent in the blood during the last half of the incubation period as the second most influential factor in the model (Figure 2 A), while using the LOW prevalence it ranks fifth (Figure 2 B). The four variables – the presence (or not) of vCJD agent in blood during the last half of incubation period (P_{LH}), adjustment for route of administration ($A_{ic\text{-}iv}$), FXI usage (D_{Tu}) (u), and FXI yield (Y_{fT}) (u/kg), do reassort and change rank when the two different prevalence estimates were used. Overall, however, they were somewhat similar in asserting their influence on the estimated risk outcome(s), but had significantly less influence when compared to that of reduction of infectivity during processing and manufacture. Although these types of sensitivity analysis and tornado plots are often used to identify influential factors of risk, their use has some limitations. Factors are examined singly or in isolation so interaction among various factors that may influence the risk estimate are not addressed.

IV. D. Uncertainty and Data Gaps

Uncertainty arises from the absence of information or availability of limited information. In our probabilistic model statistical distributions are used, where possible, to represent the uncertainty of much of the information used in the model. There are uncertainties in the information and the model that we were unable to quantify and that are not represented in the final risk estimates. Some of the difficult to quantify uncertainties are associated with the extrapolation of a human dose-response relationship based on animal data, an assumed linear dose response with no uncertainty or variability bounds, and assumption of infectivity in the last 50% of the incubation period. We express the uncertainty of the final risk estimates generated from the model using a mathematical mean (average) of exposure in ID_{50} units and the 5^{th} and 95^{th} percentiles, which represent the 90% confidence interval for each estimate. The uncertainty for the risk estimates generated by this FXI risk assessment model is significant and decision makers should use the results with caution. Similarly, patients and physicians should understand that the uncertainties are too great at this time to determine the presence, absence or degree of actual risk. In the future, additional research and information may be substituted for assumptions or used to improve estimates for the individual parameters and ultimately improve the precision of the final risk estimates generated by the model.

Even considering the associated uncertainty of estimated risks, risk assessment provides an estimate of risk based on the current and known information. It is still a useful tool that can inform the science-based decision making process. It can identify data gaps and research priorities where additional research and information would have the greatest impact on enhancing the final risk estimates. The sensitivity analysis results in Section IV.C. indicated that the risk assessment results are highly dependent upon log reduction of vCJD agent (R_{Log}) during the manufacturing process. The modeled estimates were based upon levels of reduction seen for a manufacturing step that was similar in some but not all respects to that used for FXI. More high quality data on the levels of vCJD agent clearance achieved during the FXI manufacturing would likely improve the final risk estimate generated by the FDA model. Given the lack of data on vCJD agent clearance for FXI 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.

IV. E. Conclusions

Potential exposure to the vCJD agent present in FXI manufactured in the UK and used during investigational studies in the US from 1989 to 2000 was estimated in this probabilistic risk assessment.

Although no UK-manufactured FXI product used in the US under IND from 1989 to 2000 was manufactured from "implicated" plasma pools that contained donations from an individual(s) later diagnosed with known vCJD, it is possible that FXI product manufactured from UK plasma in the 1990s may have been manufactured from plasma pools that contained a plasma donation(s) from an individual who was unknowingly incubating vCJD. The results of the computer modeling suggest that, if so, there could have been exposure to the vCJD agent and a potential risk of infection to some recipients of FXI, particularly if the incidence of unsuspected infection with vCJD in the UK is higher than scientists generally believe based on the occurrence to date of vCJD cases. Unfortunately, there are so many uncertainties that it is not possible based on available scientific information to provide an actual or precise estimate of any potential risk. Although the actual risk, if any, remains unknown, the computer model indicates that the most important factors affecting the potential for risk are the clearance of the vCJD agent though manufacturing steps, how much product individuals used, efficiency of the i.v. versus the i.c. route of exposure, and the vCJD prevalence in the UK donor population.

In considering the results of the risk assessment it is important to note that to date we are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived products, including pdFXI.. This includes patients receiving large amounts of other products manufactured from UK plasma donations over a long period of time. This observation suggests that the actual risk of vCJD infection from pdFXI is likely to be low. The absence of cases does not rule out the possibility of exposure that could potentially result in illness in some recipients at some future point in time.

Appendix A

Table A. Summary of Model Components and Inputs

Input Data and Information in the FXI – vCJD Risk Assessment			
III. A. Probability of donation containing vCJD infectivity and the total quantity of intravenous vCJD infectivity (i.v. ID_{50}) per plasma pool			
	Variable description	**Variable name**	**Numerical input / output**
A.1.	*Estimation of UK vCJD prevalence via two methods*		
A.1.a.	*Probability of vCJD-infected individual in UK population who will develop symptoms – determined by epidemiologic modeling-based prevalence estimate.*	$P_{vCJD\text{-}Epi}$	4 infections per million (95% CI: 3-6 cases per million)
A.1.a.i.	*Estimated Number of vCJD-infected individuals in UK population using recorded vCJD cases (1997and before) – 2004*) and epidemiological modeling based prevalence estimate*	$N_{vCJD\text{-}CE}$	$N_{vCJD\text{-}CE}$, is the sum of 138 reported vCJD cases, $N_{vCJD\text{-}Case}$, and the cases estimated by epidemiological modeling, $N_{vCJD\text{-}Epi}$, or an estimated 70 future cases; the sum of the expression is a total mean of 208 cases vCJD (95% CI: 148 – 328)
A.1.a.ii.	*Number of reported vCJD cases in UK population 1997 – 2004.*	$N_{vCJD\text{-}Case}$	138 cases
A.1.a.iii.	*Number of future vCJD-infected individuals in UK population based on epidemiological modeling prevalence estimate*	$N_{vCJD\text{-}Epi}$	The cases estimated by epidemiological modeling, $N_{vCJD\text{-}Epi}$, is an estimated 70 future cases
A.1.b.	*Probability of vCJD-infected individual in UK population using the surveillance prevalence estimate*	$P_{vCJD\text{-}Surv}$	237 infections per million (95%CI: 49-692) Or (1 / 4,225) (95% CI = 1 / 20,280)
A.2.	*Estimation of probability that infectivity will be present in blood (prionemia) in vCJD infected individuals at time of donation*		The vCJD agent is present in blood during the last half of the incubation period in vCJD infected individuals.
A.2 a.	*BSE cases reported in year y*	BSE_y	BSE case numbers shown in table 6.
A.2 b.	*Probability an infection occurring in year y*	$P_{infect\text{-}y}$	Based on equation: $P_{infect-y} = BSE_y / \sum_{y=1980}^{1996} BSE_y$
A.2 c.	*The incubation period of vCJD was calculated in the model using a gamma distribution represented by the expression Gamma (4.7, 3.6)*	IP_{vCJD}	IP_{vCJD} = *Gamma (4.7, 3.6)*

	Probability that the blood of an individual infected in year y will contain vCJD agent in the year 1997	P_{LH-y}	P_{LH-y} = Cumulative frequency of Gamma (4.7, 3.6), at x=2×(1997-y)
A.2 d.	*Probability of an infected individual having vCJD agent present in their blood (prionemic) in year 1997.*	P_{LH}	Based on equation: $P_{LH} = \sum_{y=1980}^{1996} P_{\inf ect-y} \times P_{LH-y}$
A.2 e.	The prevalence of prionemia among the UK population in year 1997	$P_{vCJD-LH}$	The prevalence of prionemia among the UK population for the year 1997, $P_{vCJD-LH}$, shown in the equation above is a product of the probability a person will have vCJD (P_{vCJD}) times the probability they will be prionemic, P_{LH}. The probability of vCJD occurring in the UK population was estimated for two distinctly different vCJD prevalences as described previously in section III. A. 1.
A. 3.	*Estimation of probabilities that a plasma pool contains a vCJD donation and probable number of vCJD donation per plasma pool*		
A. 3.a.	*Total number of donors per pool*	D_{Tpool}	20,000 donors or donations
A. 3. b.	*Probable number of vCJD donors or donations present per plasma pool*	D_{vCJD}	D_{vCJD} = Riskbinomial (α, β) = Riskbinomial (D_{Tpool}, $P_{vCJD-LH}$) or Riskbinomial (20000, $P_{vCJD-LH}$)
A. 3. c	*Probability a plasma pool containing any infected donor (donation)*	$P_{vCJD-pool}$	$P_{vCJD-pool}$= 1- Cumulative frequency of Binomial(D_{Tpool}, $P_{vCJD-LH}$), at x=0
A.4.	Estimation of Quantity of vCJD agent per donation and in plasma pools used in manufacturing UK FXI		
A.4.a.	*Estimated Total Infectivity (or i.c.ID_{50}) per vCJD donation*	I_D	(Also see outputs below)
A.4.a.i.	*Amount of recovered plasma per donation*	D_V	200 mls
A.4.a.ii.	*Infectivity of vCJD in infected blood per ml*	I_{bl}	Lognormal distribution Minimum = 0.1 ID_{50} 5th perc = 2 ID_{50} Median = 12 ID_{50} 95th perc = 30 ID_{50} Maximum = 1,000 ID_{50}
A.4.a.iii.	*Percentage of infectivity in plasma (ID_{50}/ml)*	I_{pl}	58%
A.4.a.iv.	*Total infectivity (or i.c.ID_{50}) per vCJD recovered plasma donation*	I_D	Total i.c.ID_{50} per vCJD donation is represented by the equation: $I_D = D_V \times I_{bl} \times I_{Pl-perc}$
A.4.a.v.	*Adjustment for intravenous route of*	A_{ic-iv}	Uniform distribution

	infection		Minimum = 1 Maximum = 10
Outputs			
A.4.a.	*Total infectivity (or i.c.ID_{50}) per vCJD donation*	$I_D = D_V \times I_{bl} \times I_{pl}$	
A.4.b.	*Total i.v. ID_{50} per plasma pool of 20,000 donors*	$T_{iv\text{-}pool} = \frac{D_{vCJD} \times I_D}{A_{ic\text{-}iv}}$	
Summary of output at this point in the model: $T_{iv\text{-}pool} = \frac{D_{vCJD} \times D_V \times I_{bl} \times I_{pl\text{-}perc}}{A_{ic\text{-}iv}}$			

B. Total i.v. ID_{50} per vial after processing / production of FXI			
Inputs			
B.1.	*Percentage of pool used to manufacture FXI*	$R_{W\%} = W_m / W_{st} \times 100\%$	16%
B.1.a.	*Weight of starting product*	W_{st}	5,000 kg
B.1.b.	*Portion removed and used to extract FXI*	W_m	800kg
B.2.	*Log reduction in ID_{50}s during processing*	R_{Log}	Triangular distribution Minimum = 0 $\log_{10}$ Most likely = 2 $\log_{10}$ Maximum = 4 $\log_{10}$
B.4.a.	*Yield of FXI per kg of plasma*	$Y_{f\text{-}kg}$	Uniform distribution Minimum = 150 u/kg Maximum = 180 u/kg
B.5.	*Vial size or # u per vial*	V_u	1,000 u
Outputs			
B.3.	*Total ID_{50} in FXI post-processing*	I_{pp}	$I_{pp} = I_{iv\text{-}pool} \times R_W \times 1 / 10^{RLog}$
B.4.	*Total yield of FXI from plasma pool*	Y_{fT}	$Y_{fT} = W_m \times Y_{f\text{-}kg}$
B.6.	*Total number vials and vial size produced*	V_T	$V_T = Y_{fT} / V_u$
B.7.	*Total ID_{50} per vial*	I_{vial}	$I_{vial} = I_{pp} / V_T$
Summary of output at this point in the model: $I_{vial} = \left[\frac{D_{vCJD} \times D_V \times I_{bl} \times I_{pl}}{A_{ic\text{-}iv}} \right] \times R_W \times 1/10^{RLog} \Big/ (W_m \times Y_{f\text{-}kg} / V_u)$			

C. Total Utilization of FXI			
Inputs			
C.1.	*Total Dose for Pre- and Post-surgical treatment with FXI*		
C.1.a.	*Prior to major Surgery - dose 20 – 50 u/kg given*	D_{Pre}	20 – 50 u/kg
C.1.b.	*Post-surgical maintenance of dose 20 – 50 u/kg given every 2 - 3 days*	D_{Post}	20 – 50 u/kg
Output			
C.1.c.	*Total Utilization of FXI*	$D_T = D_{Pre} + D_{Post}$	
C.2.	*Scenario 1: Treatment 60 Kg individual with 3,000 u FXI*		Shown in Table 8
C.3.	*Scenario 2: Treatment with 9,000 u FXI*		Shown in Table 8
C.4.	*Scenario 3: Treatment with 15,000 u FXI*		Shown in Table 8

Appendix B

Table B. Summary of Model Assumptions

Section	Variable and description	Assumptions used in the model
III.	Not applicable	
III. A.1. a.	$P_{vCJD\text{-}Epi}$ - *Probability of vCJD-infected individual in UK population who will develop symptoms – determined by epidemiologic modeling-based prevalence estimate.*	The lower prevalence estimate of vCJD in the UK population was based on Epidemiologica Modeling of predicted future cases 2004 – 2080 (Clark and Ghani, 2005) and reported vCJD cases in the UK from 1997 through 2004. Prevalence was estimated to be a mean of 4 per million.
III. A.1.a.i.	$N_{vCJD\text{-}CE}$ - *Estimated Number of vCJD-infected individuals in UK population using recorded vCJD cases (1997 – 2004*) and epidemiological modeling based prevalence estimate*	The variable, $N_{vCJD\text{-}CE}$, is the sum of 138 reported vCJD cases, $N_{vCJD\text{-}Case}$, and the cases estimated by epidemiological modeling, $N_{vCJD\text{-}Epi}$, or an estimated 70 future cases; the sum of the expression is a total mean of 208 cases vCJD (95% CI: 148 – 328)
III. A.1.a.ii.	$N_{vCJD\text{-}Case}$ - *Number of reported vCJD cases in UK population 1997 – 2004.*	Based on reported cases of vCJD from 1997 through 2004 of 138 cases (see Table 3).
III. A.1.a.iii.	$N_{vCJD\text{-}Epi}$ - *Number of future vCJD-infected individual in UK population based on epidemiological modeling prevalence estimate*	Our model uses the Clarke and Ghani (2005) estimate of 70 future cases of vCJD with a 95% confidence interval of 10 – 190 cases for the years 2005 – 2080. Assuming the population of the UK in 1997 is approximately 58 million.
III. A.1.b.	$P_{vCJD\text{-}Surv}$ - *Probability of vCJD-infected individual in UK population using the surveillance prevalence estimate*	The higher prevalence estimate of vCJD in the UK population was based on surveillance studies of tonsils and appendices (Hilton et al 2004) and assumed to be a mean of 1 in 4,225 (95% CI: 1 / 20,300 to 1 / 1,450) or 237 per million (95% CI: 49-692 per million).
III. A.2.	**Estimation of probability that infectivity will be present in blood (prionemia) in vCJD infected individuals at time of donation**	
III. A.2 a.	BSE_y-*BSE cases reported in year y*	**Data used in the model**: World Organization for Animal Health (OIE, 2006), shown in **Table 5**, was used to determine the number of cases of BSE reported in the UK. [[http://www.oie.int/eng/info/en_esbru.htm#4 (Accessed on May 30, 2006)]
III. A.2 b.	$P_{infect\text{-}y}$-*Probability an infection occurring in year y*	The probability of a vCJD infection occurring in a specific year is a function of exposure in that specific year, which is proportional to the number of BSE cases reported in that specific year (more BSE cases higher probability of getting infected) compared to the total BSE cases for all years through 1996.
III. A.2 c.	$P_{LH\text{-}y}$ – *Probability that the blood of an individual infected in year y will contain vCJD agent in the year 1997* $1P_{vCJD}$ - *The incubation period of vCJD was calculated in the model using a gamma distribution represented by the expression Gamma (4.7, 3.6)*	**Assumption 1:** FXI was made in the UK between 1989 and 1997. The model estimates the risk for using FXI made in 1997, assuming year of 1997 is the worst year because accumulation of vCJD asymptomatic individuals in the donor population. **Assumption 2:** The incubation period of vCJD can be represented by a gamma distribution expressed as Gamma (4.7, 3.6) which gives mean incubation period of 14 years and median estimated incubation period of 13 years. **Assumption 3:** The infectivity of vCJD agent present in the blood of infected individual only when the disease is at the last incubation period
III. A.2 d.	P_{LH}- *Probability of an infected individual having vCJD agent present in their blood (prionemic) in year 1997.*	The probability an individual would have been infected in year y and also have prionemia in year 1997 is the product of $P_{infect\text{-}y}$ and $P_{LH\text{-}y}$. Probability of an infected individual having vCJD agent present in their blood (prionemic) in year 1997 is the sum of this probability for any year from 1980 through 1996.
III. A.2 e.	$P_{vCJD\text{-}LH}$-The prevalence of prionemia	The probability of vCJD occurring in the UK population was

	among the UK population in year 1997 is represented by the equation: $P_{vCJD-LH} = P_{vCJD} \times P_{LH}$	estimated for two distinctly different vCJD prevalences as described previously in section III. A. 1.
III. A. 3.	**Estimation of probabilities that a plasma pool contains a vCJD donation and probable number of vCJD donation per plasma pool**	
III. A. 3. a.	D_{Tpool} - *Total number of vCJD donations per pool*	Production of FXI included the pooling of plasma donations recovered from whole blood from approximately 20,000 donations
III. A. 3. b.	D_{vCJD} -*Probable number of vCJD donors or donations present per plasma pool*	The number of vCJD donors per plasma pool is represented by a binomial distribution defined by two arguments alpha (α) and beta (β) (represented in the model by the expression Riskbinomial (α, β)). Alpha represents the probability of a donor to be prionemia when donating, which is the prevalence of prionemia among the UK population in year 1997 ($P_{vCJD-LH}$ calculated in III.A.2.e). Beta is the total number of donors per plasma pool (D_{Tpool}), which are 20,000 in this case, represented by the expression: D_{vCJD} = Riskbinomial (α, β) = Riskbinomial ($P_{vCJD-LH}$, D_{Tpool})
III. A. 3. c.	$P_{vCJD-pool}$ –*Probability a plasma pool containing any vCJD donor (donation)*	Probability a plasma pool containing any vCJD donor (donation) was: 1 minus the probability a plasma pool would contain any vCJD donor (donation). $P_{vCJD-pool}$ = 1- Cumulative frequency of Binomial(D_{Tpool}, $P_{vCJD-LH}$), at x=0
III. A.4. Estimation of Quantity of vCJD agent per donation and in plasma pools used in manufacturing UK FXI		
III. A.4.a.i.	D_V - *Amount of recovered plasma per donation*	The model assumes that approximately 200 milliliters (mls) of plasma can be separated away from the blood cells.
III. A.4.a.ii.	I_{bl} - *Infectivity of vCJD (or i.c.ID_{50}s) present in infected blood per ml*	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 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 most likely of amount 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.
III. A.4.a.iii.	$I_{Pl-perc}$ - *Percentage infectivity associated with plasma (i.c.ID_{50}/ml)*	The model uses the more conservative of the two outcomes and assumes that 58% of infectivity is associated with plasma.
III. A. 4.a.iv.	I_D - *Total infectivity (or i.c.ID_{50}) per vCJD recovered plasma donation*	One ID_{50} is the amount of material containing infectious agent that has a 50% probability of causing infection in an individual or population.
III. A. 4.a.v.	A_{it-iv} - *Adjustment for intravenous route of infection*	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.
III. A. 4.b.	$I_{iv-pool}$ - *Total intravenous infectivity or i.v.ID_{50} per plasma pool of 20,000 donors*	
III.B. Total i.v. ID_{50} per vial after processing / production of FXI		
III.B.1.a.	W_{st} - *Weight of starting product*	Weight of starting product is represented in the model by a single value point estimate of 5,000 kg.
III.B.1.b.	W_m - *800kg portion removed and used to extract FXI* $R_{W\%}$ - *Percentage of pool used to manufacture FXI*	800 kg of material was removed and used to produce FXI. Approximately 16% of starting plasma material from 20,000 donations was used in the manufacture of FXI.
III.B.2.	R_{Log} - *Log reduction in ID_{50}s during processing*	Processing reduction is represented by a triangular statistical distribution representing a reduction in ID_{50}s during processing of (0, 2,4) Log_{10} i.v. ID_{50}/ml (minimum, most likely, and maximum).

		The model assumes that infectivity is reduced but not entirely eliminated from plasma and the product during processing. Therefore, although the amount of ID_{50} vCJD agent may be reduced the percentage of pools and vials containing the agent still remains the same.
III.B.4.	Y_{FI} - *Total yield of FXI from plasma pool*	The yield of FXI per kg plasma was approximately 150 to 180 u, subsequently the model estimates the total yield of FXI as 120,000 to 144,000 u per batch of 800 kg starting material. FXI was distributed in vials of 1,000 u each.
III.C. Utilization by patients with FXI deficiency undergoing Surgery		
III.C.1.	*Total Dose for Pre- and Post-surgical treatment with FXI*	**Scenario 1** – Treatment of a 60kg individual with FXI (20 - 50 u/kg) once during or after surgery for a total patient dose of approximately 3,000 u. **Scenario 2** - Treatment of a 60kg individual both pre- and post-surgery with a total of approximately 9,000 u of FXI. **Scenario 3** - Treatment of a 60kg individual both pre- and post-surgery with a total of approximately 15,000 u of FXI.

Appendix C

Table C. Potential Probability and Number of vCJD Donations in Plasma Pool expressed with mean, median and 5th- 95th percentile values. (Expanded Table 7 from document).

	MODEL OUTPUT USING LOWER PREVALENCE ESTIMATE *vCJD Case Prevalence from epidemiological modeling ~4 per million (Clark and Ghani, 2005)*			*MODEL OUTPUT USING HIGHER PREVALENCE ESTIMATE* *vCJD Infection estimate from tissue surveillance study 1 in 4,225 (Hilton, et al 2004)*		
	Mean	**Median**	**5th- 95th percentiles[a]**	**Mean**	**Median**	**5th- 95th percentiles[a]**
Probability pool contains vCJD donation	1.6%	1.6%	1.1% -2.1%	50%	68.5%	18% - 77%
Number vCJD donations per pool	0.02	0	0 – 0[b]	0.75	1.0	0 - 3

[a] The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model are expected to fall within this defined interval at least 90% of the time.
[b] For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FXI recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FXI plasma pool would be rare and more than 90% of FXI product vials would not be predicted to contain vCJD agent.

Appendix D

Table D. –Potential Exposure and Potential Risk per Person per FXI Treatment Scenario. Hypothetical scenarios provide an estimate of the magnitude of potential exposure to vCJD agent i.v. ID_{50} and potential risk that might occur per treatment course. A treatment course might include prophylactic treatment prior to a surgery, or medical procedure and possibly several post-surgical or post-procedure treatments with FXI. (Expanded Table 8 from document to include median exposure and risk estimates).

			MODEL OUTPUT USING LOWER PREVALENCE ESTIMATE *vCJD Case Prevalence from epidemiological modeling ~4 per million (Clark and Ghani, 2005)*		***MODEL OUTPUT USING HIGHER PREVALENCE ESTIMATE*** *vCJD Infection estimate from tissue surveillance study 1 in 4,225 (Hilton, et al 2004)*	
Scenario	**Quantity FXI Utilized (u*)**	**Central tendency measure and percentiles**	**Potential exposure to vCJD i.v. ID_{50}**	**Potential vCJD risk per person**	**Potential exposure to vCJD i.v. ID_{50}**	**Potential vCJD risk per person**
Scenario 1: Treatment 3,000 u	3,000 u	Mean: Median[c]: 5th-95th perc[d]:	3.11×10^{-3} [a] 0 0 – 0[e]	1 in 643 [b] 0 0 – 0[e]	0.12[a] 0.007 0 – 0.57	1 in 17 [b] 1 in 286 0 – 1 in 3.5
Scenario 2: Treatment 9,000 u	9,000 u	Mean: Median[c]: 5th-95th perc[d]:	9.33×10^{-2} [a] 0 0 – 0[e]	1 in 214 [b] 0 0 – 0[e]	0.36[a] 0.021 0 – 1.70	1 in 5.6 [b] 1 in 95 0 – 1 in 1.2
Scenario 3: Treatment 15,000 u	15,000 u	Mean: Median[c]: 5th-95th perc[d]:	1.55×10^{-2} [a] 0 0 – 0[e]	1 in130 [b] 0 0 – 0[e]	0.59[a] 0.036 0 – 2.86	1 in 3.4 [b] 1 in 56 0 – 1 in 1

*u - represents units of FXI – and is equivalent to the term "unit" or "units" used in this document

a Mean vCJD i.v. ID_{50} (per treatment course) - the average predicted quantity of vCJD agent an individual in a specific treatment group is predicted to receive based on the model.

b Mean potential vCJD risk – the risk of potential vCJD infection based on animal model dose-response information. Mean potential vCJD risk = Total mean quantity i.v. ID_{50} (per treatment course) x 0.5 (50 % chance infection - ID_{50})

c Median – A measure of central tendency that reports the value of the exposure and risk estimate at the 50th percentile

d The 5th- 95th perc (percentiles) are the minimum and maximum numbers that define the range of values constituting the 90% confidence interval. Accordingly, the mean risk estimates generated by the model should fall within this defined interval at least 90% of the time.

e For a 5th and 95th percentile interval of 0 and 0, respectively, the model estimates that for at least 90% of FXI recipients the risk is zero. At low vCJD prevalence, donation by a vCJD infected donor to a FXI plasma pool would be rare and more than 90% of FXI product vials would not be predicted to contain vCJD agent.

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-1

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 3 月 16 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			研究報告の公表状況	Potential vCJD risk from US licensed plasma-derived Factor VIII (pdFVIII, antihemophilic factor) products www.fda.gov/cber/blood/vcjdplasma.htm	公表国 米国	
販売名（企業名）						

研究報告の概要

英国の赤血球輸血を介した vCJD 伝播例 4 例の報告を受けて，米国 FDA は同国の承認薬である血漿由来血液凝固第 VIII 因子製剤の投与を受けた血友病患者に対しリスク評価を実施した。この評価は，vCJD 誘発物質が血漿分画に存在すると仮定した場合，血漿由来第 VIII 因子製剤の原料となる血漿分画が，アルブミン，免疫グロブリン及び血液凝固第 IX 因子等，他の製剤の血漿分画よりも多く含むと考えられていることから実施された。しかしながら，血漿由来製剤の製造に用いるすべての血漿分画は，vCJD 感染物質を減少，あるいは除去する工程が導入されている。また，英国で採集された血漿由来の血液製剤の大量投与を受けた患者を含め，血液凝固因子障害をもつ患者における vCJD はこれまでに報告されていない。現時点ではこれを正確に評価できないことは明確であるが，米国公衆衛生局は，米国承認薬である血漿由来血液凝固第 VIII 因子製剤の投与を受けた患者への vCJD 感染リスクは極めて低いと推定しており，その他の血漿由来製剤によるリスクはさらに低いと考えている。しかしながら，FDA は予防措置として，BSE 及び vCJD の有病率が米国よりも大幅に上回る欧州を広範囲に旅行した供血者，又は欧州に在住していた供血者を除外するよう勧告した。また，後に vCJD と診断された供血者の血液より製造された血液成分及び血漿由来製剤については回収されることになっている。

使用上の注意記載状況・その他参考事項等

BYL-2007-0278

報告企業の意見	今後の対応
米国採取の血漿由来血液凝固第VIII因子製剤によるvCJD感染リスクは極めて低いと推定される。しかしながら，FDA は予防措置として，長期間を欧州で過ごした経験のある供血者を除外するよう勧告している。弊社の血漿分画製剤及びコージネイト FS 又はコージネイト FS バイオセットの製造工程培地で使用されている血漿分画成分は，米国採取の血漿から製造されており（万一，存在しても）感染リスクは極めて低いと考えられる。また，FDA が勧告している追加の予防措置は実施すべきであると考える。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD)

From Plasma-Derived Products

In recent years, questions have been raised concerning the potential risk of variant Creutzfeldt-Jakob disease (vCJD - a rare but fatal brain infection) for recipients of plasma- derived clotting factors, including United States (US) licensed Factor Eight (pdFVIII), Factor Nine (pdFIX), and other plasma-derived products such as immune globulins and albumin. In response to these questions, FDA conducted a risk assessment. Based on the risk assessment, the US Public Health Service believes that the risk of vCJD to patients who receive US licensed pdFVIII products is most likely to be extremely small, although we do not know the risk with certainty. vCJD risk from other plasma derived products, including Factor IX, is likely to be as small or smaller.

This web page provides FDA's risk assessment for US licensed pdFVIII and risk communication materials for this product and other plasma derivatives. Included are Key Points, and Questions and Answers. Additional links are provided to FDA's current guidance documents on deferral of blood and plasma donors who may be at increased risk of vCJD, and to other sources of information regarding vCJD.

Documents Regarding US Licensed pdFVIII, and Other US Licensed Plasma Derivatives Including pdFIX

- Potential vCJD Risk From US Licensed Plasma-Derived Factor VIII (pdFVIII, Antihemophilic Factor) Products: Summary Information, Key Points
- Risk Assessment (PDF, 582 KB)
- Risk Assessment Appendix (PDF, 623 KB)
- Questions and Answers on vCJD and pdFVIII
- Questions and Answers on vCJD and Plasma Derivatives Other than pdFVIII

Guidance on Donor Deferral Related to CJD and vCJD

- Draft Guidance for Industry: Amendment (Donor Deferral for Transfusion in France Since 1980) to "Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products" - 8/2006
- Questions and Answers on FDA Guidance: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob (CJD) Disease and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/22/2004
- Guidance for Industry: Revised Preventive Measures to Reduce the Possible Risk of Transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by Blood and Blood Products - 1/2002

Other Sources of Information

- Transmissible Spongiform Encephalopathies Advisory Committee
- Blood Products Advisory Committee Meeting – Summary of Recent TSEAC Meeting and Statement about FXI from the UK, on October 21, 2004
- Information on vCJD: Centers for Disease Control and Prevention
- Information on Bovine Spongiform Encephalopathy ("Mad Cow Disease"): US Department of Agriculture

Patient Organizations:

- Committee of Ten Thousand
- Hemophilia Federation of America
- National Hemophilia Foundation and/or HANDI
- World Federation of Hemophilia

Updated: March 15, 2007

医薬品
医薬部外品　研究報告　調査報告書
化粧品

別紙 3-2

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研究報告の概要

本文献では米国で牛海綿状脳症（BSE）関連の診断を受けた 2 症例（2003 年の報告例：症例 1，2004 年の報告例、症例 2 の特徴について報告する。両症例の脳切片について免疫組織化学検査及びウェスタンブロット法を実施したところ，微妙な違いが明らかになった。症例 1 では脳幹の閂領域に海綿状変性及び広範な異常プリオン蛋白（PrP^{Sc}）の異常沈着がみられた一方，症例 2 では空胞状の変化はみられず PrP^{Sc} はそれほど多くなかった。ウェスタンブロット法によって，症例 1 及び 2 の PrP^{Sc} は主として 2 つの特異的なモノクローナル抗体に反応したことが示された。また，症例 2 の PrP^{Sc} は高分子量に位置する異常な電気泳動パターンを示した。両症例のプリオン蛋白遺伝子の配列を決定したところ，ウシについて過去に報告された種々の配列に当てはまることが示されたため，生殖細胞突然変異によって当該疾患が誘発される可能性は低い。症例 2 に認められた異常な特性は「高分子量 BSE」又は H 型 BSE に一致し，高分子量の非グリコシル化 PrP^{Sc} を示す。現在のところ，H 型 BSE が報告されているのはフランス及びドイツのみである。

使用上の注意記載状況・その他参考事項等

BYL-2007-0284

報告企業の意見	今後の対応
米国で報告された BSE の最初の 2 例の分子特性は BSE 誘発物質の 2 つの異なる株により引き起こされやすく，実質的に「散発性」BSE の仮説が除外されることを示唆するものである。興味深いことに，1 例では H 型 BSE である可能性が高く，欧州以外では過去に報告がなかったものである。以前に報告された米国農務省当局によるリスク評価では，米国の BSE 発症のリスクが増大することはほとんどないと結論付けられた。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

J Vet Diagn Invest 19:142–154 (2007)

Identification and characterization of two bovine spongiform encephalopathy cases diagnosed in the United States

Jürgen A. Richt,[1] Robert A. Kunkle, David Alt, Eric M. Nicholson, Amir N. Hamir, Stefanie Czub, John Kluge, Arthur J. Davis, S. Mark Hall

Abstract. Bovine spongiform encephalopathy (BSE) is a transmissible spongiform encephalopathy of cattle, first detected in 1986 in the United Kingdom and subsequently in other countries. It is the most likely cause of variant Creutzfeldt-Jakob disease (vCJD) in humans, but the origin of BSE has not been elucidated so far. This report describes the identification and characterization of two cases of BSE diagnosed in the United States. Case 1 (December 2003) exhibited spongiform changes in the obex area of the brainstem and the presence of the abnormal form of the prion protein, PrP^{Sc}, in the same brain area, by immunohistochemistry (IHC) and Western blot analysis. Initial suspect diagnosis of BSE for case 2 (November 2004) was made by a rapid ELISA-based BSE test. Case 2 did not exhibit unambiguous spongiform changes in the obex area, but PrP^{Sc} was detected by IHC and enrichment Western blot analysis in the obex. Using Western blot analysis, PrP^{Sc} from case 1 showed molecular features similar to typical BSE isolates, whereas PrP^{Sc} from case 2 revealed an unusual molecular PrP^{Sc} pattern: molecular mass of the unglycosylated and monoglycosylated isoform was higher than that of typical BSE isolates and case 2 was strongly labeled with antibody P4, which is consistent with a higher molecular mass. Sequencing of the prion protein gene of both BSE-positive animals revealed that the sequences of both animals were?within the range of the prion protein gene sequence diversity previously reported for cattle.

Key words: Bovine spongiform encephalopathy; cattle; immunohistochemistry; *Prnp* gene; Western blot.

Introduction

Transmissible spongiform encephalopathy (TSE) agents or prions induce fatal neurodegenerative diseases in humans and in other mammals. They are transmissible among their species of origin, but they can also cross some species barriers and induce infection with or without disease in other species. Human TSEs include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker syndrome, Kuru, and fatal familial insomnia.[38] In animals, 4 distinct TSE diseases are recognized: scrapie in sheep and goats, transmissible mink encephalopathy (TME) in mink, chronic wasting disease (CWD) in cervids, and bovine spongiform encephalopathy (BSE) in cattle. BSE is transmissible via BSE-contaminated feed to cats (feline spongiform encephalopathy, FSE) and exotic ungulates (exotic ungulate encephalopathy, EUE).[50,51]

Prions are proteinaceous infectious particles and are the causative agents of TSEs. They are host-coded proteins that have undergone conformational changes and have biological and physicochemical characteristics that differ significantly from those of other infectious agents. For example, they are resistant to inactivation processes that are effective against conventional viruses including those that alter nucleic acid structure or function. These include ionizing and UV radiation,[1] and inactivation by formalin.[21] In contrast, infectivity is highly susceptible to procedures that modify protein conformation.[41] In TSE disease, the normal cellular protein, PrP^{C}, is converted to abnormal prion protein, PrP^{Sc}. PrP^{Sc} exhibits increased beta sheet content, a change that may drive the additional changes in solubility and protease resistance.[40] Unlike normal cellular protein, PrP^{Sc} is relatively insoluble in detergents, is relatively resistant to proteases,[39] and is capable of causing a conformational change in additional molecules of PrP^{c}. The precise function of the normal PrP^{c} in healthy animals remains unknown. There is some evidence to show that PrP^{c} might play a role in sleep physiology, in

From the Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa, 50010 (Richt, Kunkle, Nicholson, Hamir), the Bacterial Diseases of Livestock Research Unit, National Animal Disease Center, USDA, Agricultural Research Service, Ames, Iowa, 50010 (Alt), the Pathobiology Laboratory, National Veterinary Service Laboratory, USDA, Animal and Plant Health Inspection Agency, Ames, Iowa, 50010 (Kluge, Davis, Hall), and the National BSE Reference Laboratory, Canadian Food Inspection Agency, Winnipeg, Manitoba R3M 3M4, Canada (Czub).

[1] Corresponding Author: Jürgen A. Richt, DVM, PhD, National Animal Disease Center, PO Box 70, Ames, IA 50010, USA. jricht@nadc.ars.usda.gov

resistance to oxidative stress, in signal transduction, and in self-renewal of hematopoietic stem cells.[17,31,33,55]

TSE disease involves the accumulation of PrP^{Sc} in the central nervous system (CNS) of the host, eventually leading to neurodegeneration and disease. In TSE-affected animals, PrP^{c} has a determinant role in the incubation time and species barrier.[8] Mice lacking prion protein gene (*Prnp*) expression are not susceptible to TSE agents or prion infection, demonstrating the key role of PrP^{c} in TSEs.[8] Susceptibility to prions thus depends upon the presence of PrP^{c} on the cell membrane of the host; prions do not propagate in brains that lack PrP^{c}.[6]

BSE first emerged in 1986 in the United Kingdom, where more than 180,000 cases have been diagnosed to date (August 2006). Widely referred to as "mad cow disease", BSE subsequently spread to many countries, predominantly in Western Europe. These outbreaks, caused by the consumption of infected meat and bone meal containing the malformed prion protein, have resulted in the destruction of thousands of cattle and have caused significant economic losses. BSE is a chronic degenerative disease affecting the central nervous system of cattle. Affected animals display changes in temperament, abnormal posture, incoordination and difficulty in rising, decreased milk production, and/or loss of body weight despite continued appetite.[42] The average incubation period is about 4–6 years and all affected animals succumb to the disease.[30] Following the onset of clinical signs, the animal's condition deteriorates until it either dies or is destroyed. This process usually takes from 2 weeks to 6 months. Most cases in the United Kingdom occurred in dairy cows between 3 and 6 years of age, with the highest susceptibility to infection being in the first 6 months of life; adult cattle appear to be at relatively low risk of infection.[3]

Epidemiological surveillance programs carried out in many European and non-European countries have discovered BSE-positive animals within the last decade.[18,36] In May 2003, Canada reported its first indigenous case of BSE, detected as part of the Canadian BSE surveillance program on a commercial cow-calf operation in Alberta, Canada [13,45]. Since then, Canada has reported 7 additional cases of BSE (two in 2005, five in 2006 – as of August 2006), all of them detected as part of the ongoing Canadian BSE surveillance and the majority of cases stemming from a distinct area in Alberta.

All currently validated diagnostic tests for BSE require brain tissue.[35,49] There is currently no validated *ante mortem* test for BSE. The original diagnostic test method was histopathology in which brain sections exhibiting the classical vacuoles and spongiform changes in specific areas are used for diagnosis.[35] In the mid-1990s, immunohistochemistry (IHC) and Western blotting were developed for the detection of PrP^{Sc} in tissues.[35] Both IHC and Western blot are considered confirmatory tests for BSE by the World Organization for Animal Health (OIE).[35] In the past decade, "rapid tests" have been introduced commercially for BSE surveillance.[35]

This report describes the identification and characterization of 2 BSE cases diagnosed in the United States. The first US BSE case (December 2003) was identified by using the BSE surveillance system that was in place in the United States from May 1990 until May 2004. Using this system, brainstem samples from field cases of cattle exhibiting signs of neurologic disease, cattle condemned at slaughter for neurologic reasons, rabies-negative cattle submitted to public health laboratories, neurologic cases submitted to veterinary diagnostic laboratories and veterinary teaching hospitals, and sampling of cattle that were nonambulatory (downer cattle/fallen stock) were sent to the National Veterinary Service Laboratory (NVSL) for confirmatory IHC testing. Before 1995, BSE testing at the NVSL was done by histological analysis of brain sections for the presence of BSE-typical spongiform lesions. After 1995, testing included the use of the newly established IHC procedures for the detection of PrP^{Sc} in brain sections of TSE-infected animals.[30] After the first case of BSE in the United States was diagnosed, an enhanced BSE surveillance program was established on June 1, 2004, based on the use of a rapid screening test[a], followed by confirmatory testing (IHC and/or Western blot, since June 2005 both methods are used in parallel) for any reactive sample, designated "inconclusive." As of August 6, 2006, more than 775,000 samples have been tested and 2 samples were found to be BSE positive. Currently, an "inconclusive" sample is defined as being positive by the initial one-well ELISA and again positive in at least 1 out of 2 wells in a repeat test of the original homogenate. USDA has designed its enhanced testing program to collect the majority of samples from high-risk animals in the following categories: nonambulatory cattle, cattle exhibiting signs of a central nervous system disorder, cattle exhibiting other signs that may be associated with BSE, such as emaciation or injury, and dead cattle. In addition, all cattle condemned on *ante mortem* inspection are sampled. In this report, detection of PrP^{Sc} by immunohistochemistry and Western blot analyses are presented. Brain material from both cases is compared with each other and with well-defined BSE isolates from other countries. In addition, sequences of the prion protein gene of both BSE-positive animals are discussed.

Material and methods

Animals and tissues

Case 1 was an approximately 6.5-year-old, nonambulatory cow, slaughtered in December 2003 in Moses Lake, Washington State. The animal was imported from Canada in 2001. Case 2 was a downer cow, approximately 12 years old, and sent to a pet food plant in November 2004. The animal was born and raised in Texas. Brain samples were taken from both animals according to the USDA BSE surveillance plan and shipped to the National Veterinary Service Laboratories (NVSL) in Ames, Iowa. The obex sample from Case 2 was found to be reactive twice in a rapid BSE test performed by the Texas Veterinary Medical Diagnostic Laboratory at Texas A&M University, College Station, TX, before being shipped to NVSL. The rapid BSE test[a] used in the United States has been validated by the European Union, Canada, and the United States and has demonstrated both high sensitivity and specificity for detecting BSE prion protein in cattle. Frozen and formalin fixed samples from the medulla oblongata and the cerebellum were available for analysis. No portion of either cow's carcass entered the animal or human food chain.

Histopathology

Brain tissue was fixed in 10% buffered formalin, embedded in paraffin wax, sectioned at 5 micron thickness, and stained with hematoxylin and eosin (HE) for light microscopic examination.

Immunohistochemistry (IHC)

Brain tissue was placed in 10% buffered formalin and after a minimum of 4 days of fixation appropriate sections of obex were put in cassettes and kept in fresh formalin until they were processed for routine paraffin embedding. Using automated tissue processing, tissues were cut at 5 micron thickness and mounted onto charged glass slides[b]. Slides were set upright, drained and air-dried for a minimum of 3 hr, followed by 15 min at 80°C. The slides were then deparaffinized (xylene, ethyl alcohol gradient, distilled H_2O). Additionally, 5-micron sections of US BSE case 2 were treated with 95% formic acid for 5, 15, and 30 min at room temperature. Slides were incubated with a target retrieval solution[c], placed in a medical decloaking chamber[d], autoclaved for 30 (case 1) or 30 and 45 (case 2) min at 121°C, and then cooled for 25 min. Slides were soaked for a minimum of 5 min in Ventana APK Wash Solution[e]. The Ventana NexES carousel[e], filled with APK Wash Solution[e] and Liquid Coverslip bottles[e], was used with the PrP-specific antibody F99/97.6.1[f] at a concentration of 10 µg/ml. Slides were processed to completion using alkaline phosphatase red paraffin protocols as suggested by the manufacturer[e]. Then, slides were removed, placed in a rack and dipped thirty times in 250 ml of warm soapy tap water containing 2–3 drops liquid dishwashing detergent[g]. Afterwards, slides were rinsed in running tap water for 2 min and dehydrated in ethyl alcohol and xylene. Coverslips were added to slides and IHC results were interpreted as follows: 1) positive for PrP^{Sc}: pink to red and 2) background and negative for PrP^{Sc}: only blue background. It should be noted that the IHC procedure did not incorporate a proteinase K digestion step. For the purpose of simplicity of nomenclature, the term "PrP^{Sc}" is used to describe the abnormal prion protein. As positive controls, slides from the brainstem of a BSE-positive cow, obtained from the United Kingdom, and from the brainstem of a scrapie-positive sheep were used for Case 1; slides from brainstem of a BSE-positive cow obtained from the United Kingdom and from the US BSE Case 1 were used for Case 2. As negative controls, slides from brainstem material of BSE-negative cattle and scrapie-negative sheep were used.

Western blot analyses

Brain homogenates from US BSE case 1 were prepared from approximately 0.5 gram brainstem material and analyzed using the Prionics®-Check Western Kit[h] as suggested by the manufacturer with minor modifications regarding the detection system. It should be noted that the Prionics®-Check Western Kit[h] method is not a confirmatory assay for BSE. Samples were homogenized at room temperature with homogenization buffer[h] (10% w/v) using a PowerGen125 homogenizer[i] with a disposable probe[i] (5 times, 30 sec), and digested with proteinase K (PK)[h] for 40 min at 48°C. PK-digestion was stopped according to the manufacturer's protocol and 10–15 µl volume of sample was loaded onto pre-cast sodium dodecyl sulfate (SDS)-12% polyacrylamide gel electrophoresis (PAGE) gels[j]. SDS-PAGE was performed as described by the manufacturer and the proteins transferred from the gel to a polyvinylidene difluoride (PVDF)[k] membrane with transfer buffer[j]. The membranes were blocked with PVDF blocking buffer[j] and either incubated with antibody 6H4[h] (1:10,000 dilution or 0.1 µg/ml of a mouse IgG1 monoclonal antibody antiserum raised against human residues 144–152 [DYEDRYYRE] of the PrP peptide) or antibody P4[l] (1:10,000 dilution or 0.1 µg/ml of a mouse monoclonal antibody raised against synthetic ovine PrP residues 89–104 [GGGGWGQGG-SHSQWNK] of the PrP peptide) for 1 hr at 37°C or overnight at 4°C. After three washes in Tris-Buffered Saline Tween®-20 (TBST)[j], the membranes were incubated for 30 min at room temperature with a biotinylated sheep anti-mouse antibody[m] (1:10,000 dilution or 0.05 µg/ml in TBST). After 3 washes in TBST[j], a streptavidin-horseradish peroxidase conjugate[m] was added for 30 min. After another round of washes with TBST, bound antibodies were detected by using the ECL Plus[m] chemiluminescent substrate.

For US BSE case 2, the Prionics®-Check Western Kit[h] and the OIE-recommended Scrapie Associated Fibril (SAF)-Immunoblot method (http://www.oie.int/eng/normes/mmanual/ A_summry.htm) were used[35] with minor modifications. In contrast to the Prionics®-Check Western[h] method, the SAF Immunoblot method enriches brain samples for PrP^{Sc} by ultracentrifugation prior to loading them onto a SDS-PAGE gel. The SDS-PAGE electrophoresis conditions, subsequent transfer and immunodetection of PrP^{res} were carried out as described above. Therefore, only the enrichment method will be described in more detail. Material for analysis of case 2 was taken from the brainstem (2 g) and cerebellum (2 g) area and minced with

a new blade after removal of dura mater. A 10% (w/v) tissue homogenate in 10 mM Tris, pH 7.5, containing 5 mM $MgCl_2$ was prepared using a homogenizer[g] with a disposable probe[g] (5 times, 30 sec). The homogenate was mixed well and then again sonicated for 30 sec on ice bath (5–10 times). Benzonase®[n] was added to the mixture for a final concentration of 100 U/ml and incubated for 1 hr at 37°C while shaking. An equal volume of 20% (w/v) N-lauroylsarcosine[o] in 10 mM Tris, pH 7.5 and 1 mM DTT[o] was added to each homogenate, vortexed for 1 min every 10 min for a total of 30 min at room temperature. Homogenates were transferred to polyallomer tubes[p] and centrifuged at 20,000 × *g* for 25 min at 10°C. Supernatant was centrifuged again using polyallomer tubes[p] at 200,000 × *g* for 55 min at 10°C. The resultant supernatant was discarded, the pellet was resuspended in sterile, distilled H_2O (1 µl/mg tissue equivalent) and sonicated until suspended. Sample was split into 2 aliquots into micro-centrifuge tubes and one sample was treated with PK[q] (concentration 0.4 U/ml) by incubation at 37°C for 60 min with agitation while the control sample was not treated with PK. Phenylmethylsulphonyl fluoride[r] (PMSF) was added to a final concentration of 5 mM, incubated on ice for 15 min and transferred to a new 1.5-ml ultracentrifuge tube. Volume was brought up to 500 µl with H_2O and centrifuged at 200,000 × *g* for 1 hr at 10°C. Pellet was resuspended in SDS-PAGE sample buffer to at least 10 mg tissue equivalent per µl. Samples were sonicated on wet ice before loading on SDS-PAGE gel. For both Western blot techniques, detection was performed either on Biomax films[s] or scanned images were obtained with a Typhoon[m] imaging system. As positive control samples, BSE-positive brain material from Canadian and Swiss cattle, cattle-and sheep-passaged scrapie and CWD-positive elk and mule deer material were used for Case 1, brain material from Case 1 and sheep scrapie were used for Case 2. As negative controls, brain material from a BSE-negative cow was used.

DNA isolation and PCR amplification

Genomic DNA was extracted from 200 µl of a 10% brain homogenate (cerebellum) using the DNeasy™ tissue kit[t] according to the manufacturer's instructions. PCR was performed in a 100 µl final reaction volume containing 0.2 pmole of forward primer (5'-CAT ATG ATG CTG ACA CCC TC -3'), 0.2 pmol of reverse primer (5'-AGA AGA TAA TGA AAA CAG GAA G -3') 1× Easy-A PCR buffer, 2.5 mM $MgCl_2$, 0.8 mM each deoxyribonucleotide triphosphate (dNTP Master Mix)[u], 2.5 U of Easy-A™ high-fidelity cloning Taq DNA polymerase[v], and 0.4 ug of total DNA. Amplification was performed with the following conditions: 94°C for 5 min, followed by 30 cycles of 94°C for 30 sec (denaturation), 59°C for 30 sec (annealing), and 72°C for 1 min. A final extension step at 72°C was performed for 10 min. Amplified DNA product was purified using a GENECLEAN® spin kit[w] and sequencing was performed using the ABI 3700 DNA sequencer with the cycle sequencing kit[x]. The fragment was sequenced in duplicate using the original 2 primers and 2 internal primers 4142 and 9612 [4] for a total of 8 reactions. Databases were searched using standard nucleotide-nucleotide BLAST at the National Center for Biotechnology Information Web Site (http://www.ncbi.nlm.nih.gov). The database is a collection of sequences from several sources, including GenBank and Reference Sequence. The nucleotide sequences of cases 1 and 2 were aligned using both CLUSTAL V[26,27] and CLUSTAL W[48] with the following GENBANK accession numbers: AY335912 (bovine), AY367641 (bovine), AF016228 (elk), AY275712 (white-tailed deer), AF166334 (sheep), AJ567986 (sheep), and the Canadian BSE case using Lasergene version 5.07 software (DNASTAR-Madison WI).

Results

ELISA results

When brainstem samples from case 1 and case 2 were repeatedly tested for the presence of PrP^{Sc} using a validated ELISA test system, the mean optical density (OD) values were 1.86 for case 1 ($n = 2$) and 2.49 for case 2 ($n = 6$).

Histological and immunohistochemical examination

Brainstem samples at the level of obex were available from both cases and analyzed for the presence of spongiform changes and deposition of PrP^{Sc}. Lesions of spongiform encephalopathy diagnostic for BSE were detected in the obex region of case 1 (Fig. 1A, and were not present in the obex of case 2 (Fig. 1C). The tissue of case 2 had been frozen and therefore artifactual changes were present, but definitive lesions of BSE were not observed (Fig. 1C). In both cases the medulla at the level of the obex was examined. In case 1 there was neuropil vacuolation present in several areas and occasional scattered neuronal vacuolation. The neuropil vacuolation was most pronounced in the solitary nucleus and tract, the spinal nucleus and tract of the trigeminal nerve, the olivary nuclei, and less pronounced in the motor nucleus of the vagus. In case 2 there was a freezing artifact that precluded definitive histological interpretation of vacuolar changes, however overt TSE related vacuolar lesions were not observed. When IHC was performed, both cases were positive for the presence of PrP^{Sc}. Whereas significant amounts of PrP^{Sc} were detected in the obex area of case 1 (Fig. 1B), only weak staining for PrP^{Sc} was observed in the obex area of case 2 (Fig. 1D). Initially, the IHC of case 2 was negative, however, after formic acid treatment and extended antigen retrieval the case was positive by IHC (Fig. 1D). Extended antigen retrieval (up to 45 minutes) and the treatment of the slides with formic acid prior to immunostaining were necessary to obtain unambiguous PrP^{Sc} signals. The distribution of PrP^{Sc} in brainstem of case 2 was not as uniform nor as intense as seen with case 1 (Fig. 1B). Case 1 had intense widespread immunostaining

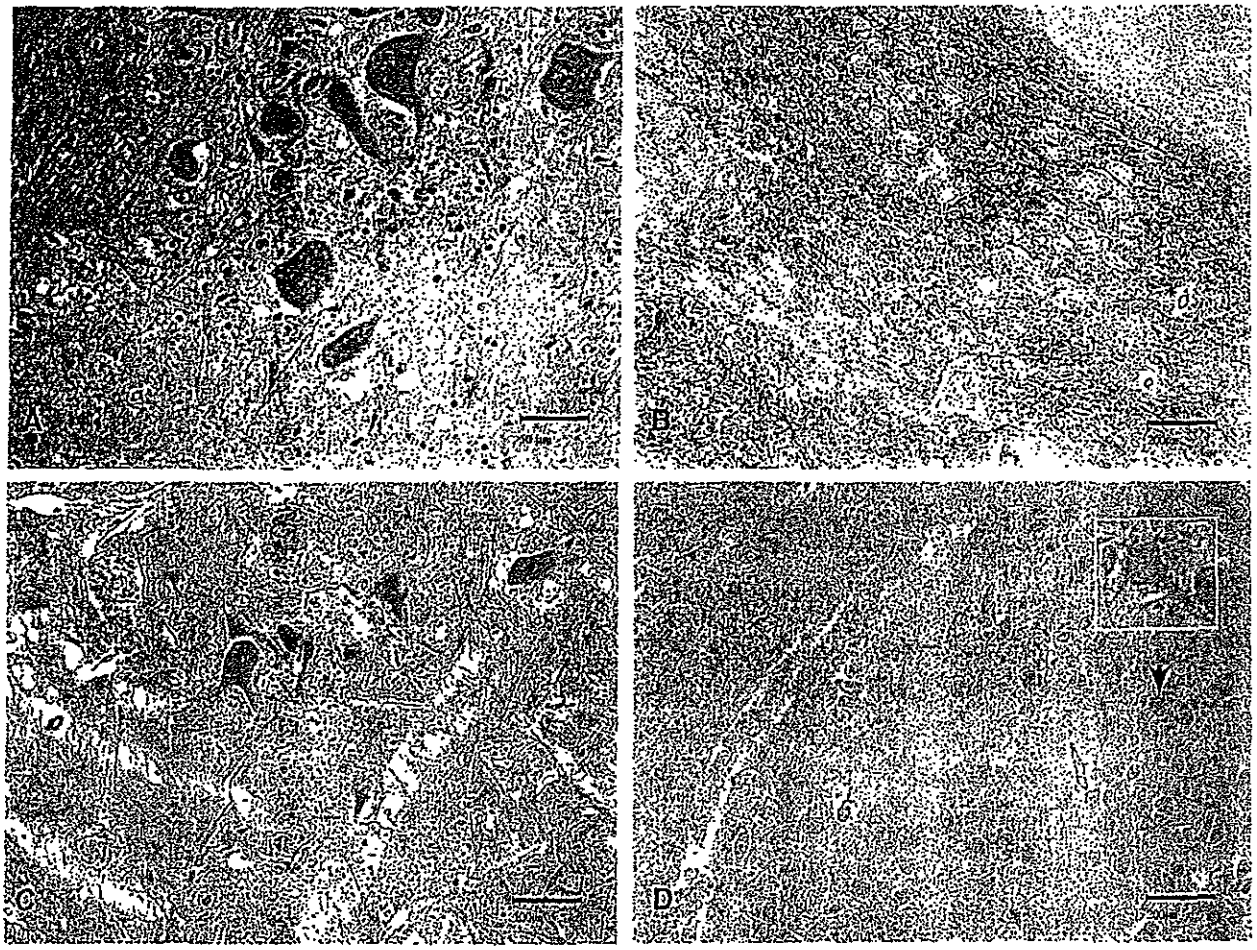

Figure 1. Histopathology and IHC on brain samples from US BSE cases. In both cases the medulla at the level of the obex was examined. US BSE case 1 is depicted in A, and B, US BSE case 2 is depicted in C, D, A; C = HE staining; B, D = IHC for PrP^{Sc} in red, counterstain with hematoxylin in blue; Arrow = Neuron surrounded by PrP^{Sc}; Inset = magnification of neuron depicted by arrow. Bar = 75 μm.

throughout the obex. This included most nuclei and gray mater neuropil. Both intracellular and intracellular staining was present. Case 2 also had widespread neuropil and intracellular immunostaining however the staining was markedly less intense than case 1 and more prominent in the lateral and ventral portions of the section as compared to case 1. The staining was most pronounced in the nucleus of the solitary tract and the nucleus of the spinal tract of the trigeminal nerve as well as the olivary nuclei.

Western blot analysis

Western blot analysis (employing the Prionics®-Check Western Kit®) of brainstem homogenates of case 1 revealed a strong positive reaction at 1 mg tissue equivalent (Fig. 2A–C). All 3 isoforms of PrP^{SC} were easily detected using the monoclonal antibody 6H4 and showed similar molecular masses when compared to the May 2003 Canadian and a Swiss BSE isolate (Fig. 2B). The glycoform profile of the 3 PrP^{Sc}-isoforms of case 1 was determined by analyzing 5 independent Western blots using the Typhoon™ imaging system employing software ImageQuant 5.2™. The diglycosylated isoform was the most prominent isoform (72.0% ± 4.7), followed by the monoglycosylated (23.3% ± 3.6) and the unglycosylated (4.0% ± 1.2) isoforms. The unglycosylated isoform of case 1 showed a lower molecular weight when compared with mule deer CWD, sheep-passaged mule deer CWD, elk CWD and sheep scrapie samples (Fig. 2A, B). Using a hybrid Western blot employing monoclonal antibodies P4 and 6H4,[44] all TSE-infected samples reacted well with antibody 6H4, however, US BSE case 1 and a cattle-passaged scrapie isolate did not react with antibody P4 (Fig. 2C, D). Sheep scrapie and mule deer CWD

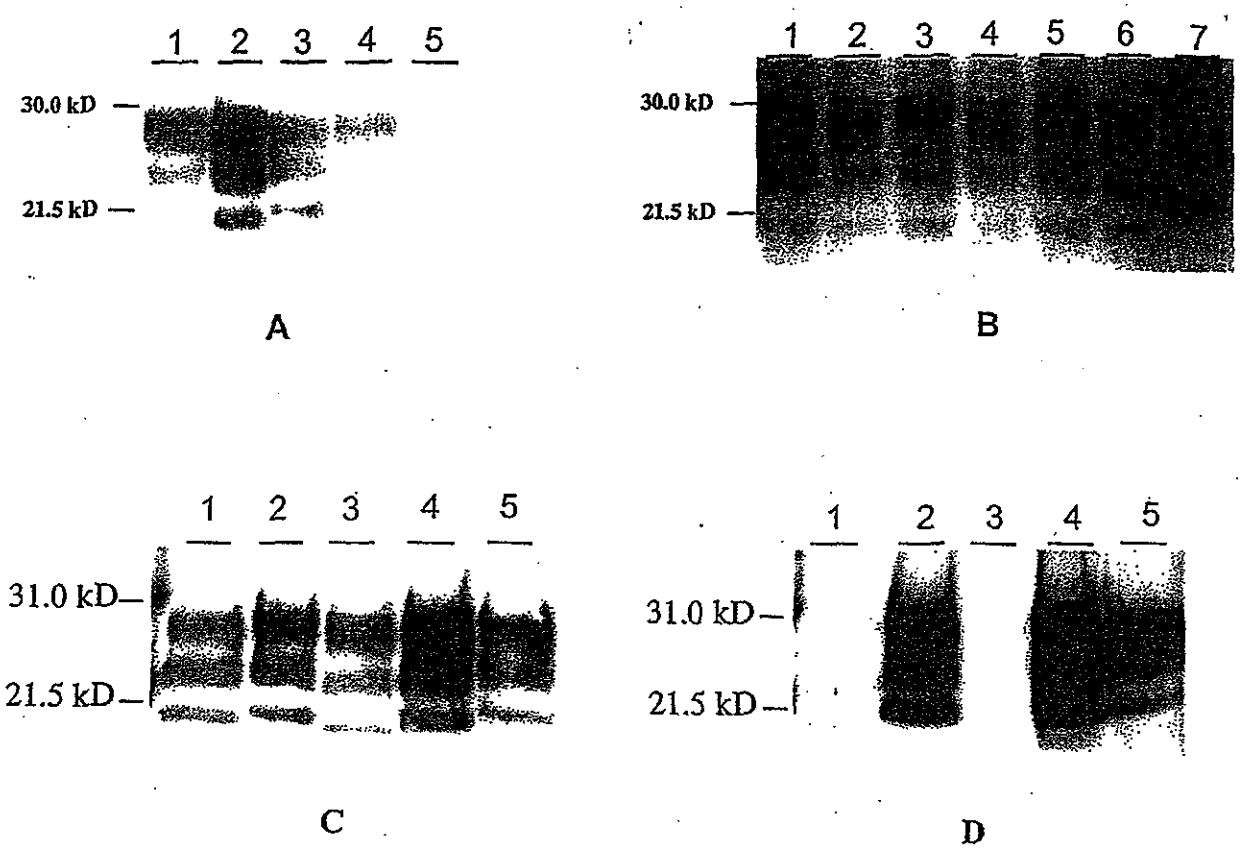

Figure 2. Western blot analysis of US BSE case 1. A, Antibody 6H4. All samples were loaded with 1-mg brain tissue equivalent. 1 = US BSE case 1; 2 = Sheep scrapie; 3 = CWD mule deer; 4 = sheep-passaged CWD mule deer; 5 = negative cattle CNS control. B, Antibody 6H4. All samples were loaded at 1-mg brain tissue equivalent. 1 = Canadian BSE isolate 2003; 2 = US BSE case 1; 3 = Canadian BSE isolate 2003; 4 = US BSE case 1; 5 = Swiss BSE isolate; 6 = US BSE case 1; 7 = elk CWD. C, and D, Hybrid Western blot analysis [44] using antibodies 6H4 (C) and P4 (D). One PAGE gel was loaded with 2 sets of identical samples (1-mg brain tissue equivalent) and cut in the middle before incubation with the respective antibodies. 1 = cattle-passaged scrapie; 2 =sheep scrapie; 3 = US BSE case 1; 4 = sheep scrapie; 5 = mule deer CWD. D. 1 = cattle-passaged scrapie; 2 = sheep scrapie; 3 = US BSE case 1; 4 = sheep scrapie; 5 = mule deer CWD.

reacted with both antibodies. As already discussed above (see Fig. 2A), the relative molecular mass of the unglycosylated isoform of the mule deer CWD isolate and the two sheep scrapie isolates was higher than the respective isoform of case 1 (Fig. 2C).

Brain material (brainstem and cerebellum) from case 2 was analyzed by Western blot with and without enrichment of PrP^{Sc}. It should be noted that it was not possible to determine the precise anatomical location where the samples were taken, since the material was frozen and thawed several times before Western blot analysis was performed. Nonenriched samples (Prionics®-Check Western Kit[f]) were analyzed at a concentration of 1–1.5 mg brain tissue equivalent (mg eq) and enriched samples (SAF Immunoblot) at a concentration of 10–150 mg eq. As shown in Fig. 3A, brainstem from case 2 was definitely positive after enrichment (≥20 mg eq), whereas the nonenriched sample (1 mg eq) was inconclusive. All 3 isoforms of PrP^{Sc} were definitely present at 20 mg eq with a strong reaction at 40 and 80 mg eq (Fig. 3A). In addition, brainstem and cerebellum samples were taken from different regions of the respective tissues and analyzed by enrichment Western blot. However, only 2 out of 4 cerebellum and 3 out of 6 brainstem samples were positive at 20 mg eq (data not shown). Glycoform profile analysis (analyzing 4 independent Western blots) revealed a significant proportion of the diglycosylated isoform (61.2% ± 2.9), and lesser amounts of the monoglycosylated (29.8% ± 2.3) and unglycosylated (9.0% ± 2.5) isoforms. Furthermore, cerebellum and brainstem samples from case 1 and case 2 were compared side by side employing monoclonal antibody 6H4. All of these samples, except for the sheep scrapie control sample, were enriched for PrP^{Sc}. The unglycosylated and monoglycosylated isoforms of PrP^{Sc} from case 2 migrated with an apparent molecular mass higher than the respective

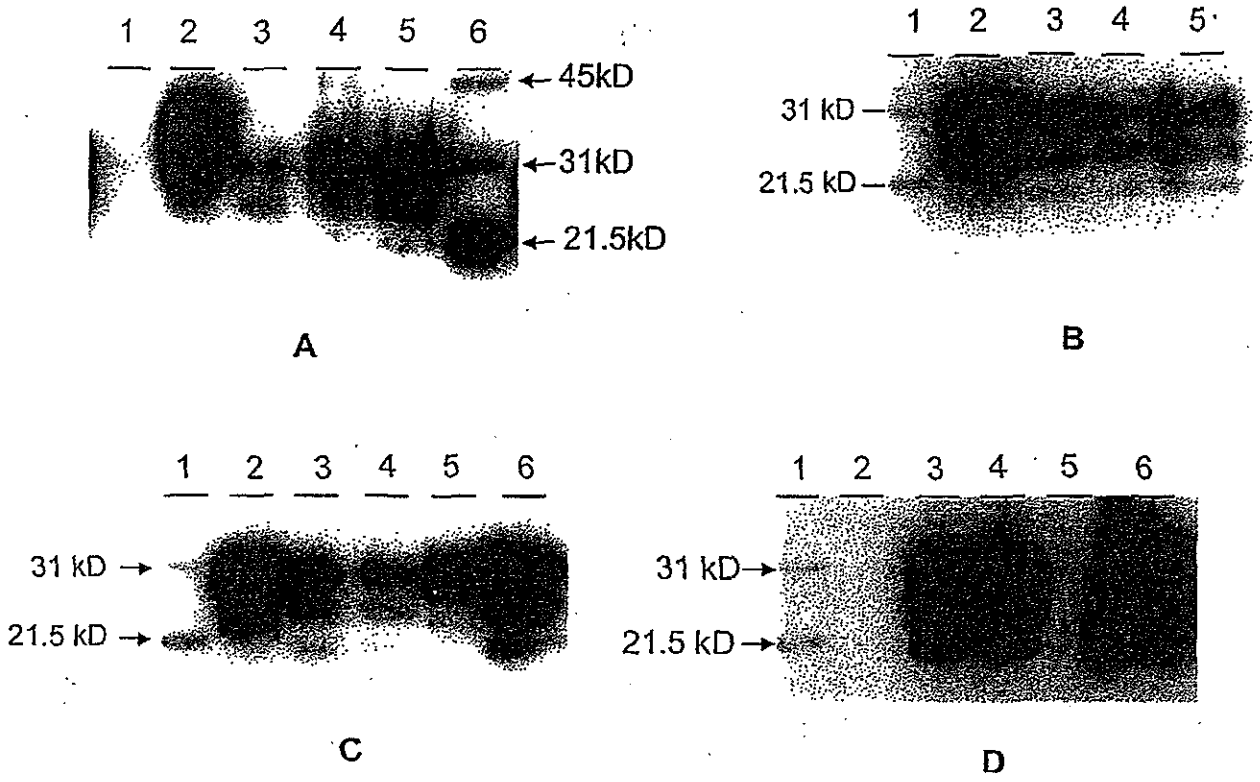

Figure 3. Western blot analysis of US BSE case 2. A, Brainstem material, antibody 6H4. 1 = US BSE case 2, 1 mg eq, PK-treated; 2 = US BSE case 2, 20 mg eq, non-PK-treated; 3 = US BSE case 2, 20 mg eq, PK-treated; 4 = US BSE case 2, 40 mg eq, PK-treated; 5 = US BSE case 2, 80 mg eq, PK-treated; 6 = Molecular weight marker™. B, Brainstem material, antibody 6H4. 1 = Molecular weight marker™; 2 = US BSE case 1, 20 mg eq; 3 = US BSE case 2, 65 mg eq; 4 = US BSE case 2, 25 mg eq; 5 = sheep scrapie, 1.5 mg eq. C, and D, Hybrid Western blot analysis [44] with cerebellum samples using antibodies 6H4 (C) and P4 (D). One PAGE gel was loaded with 2 sets of identical samples and cut in the middle before incubation with the respective antibodies. 1 = Molecular weight marker™; 2 = US BSE case 1, 3 mg eq; 3 = US BSE case 2, 50 mg eq; 4 = US BSE case 2, 50 mg eq; 5 = US BSE case 1, 3 mg eq; 6 = sheep scrapie, 1.5 mg eq.

isoforms of case 1 (Fig. 3B, 3C), indicating a relative difference in molecular mass between the two BSE cases. The reaction pattern was very similar, independent of the region of the brain (brainstem or cerebellum) used for analysis (Fig. 3B, 3C). In hybrid Western blots, enriched cerebellum samples from case 1 reacted strongly with antibody 6H4 (Fig. 3C) and either weakly or not at all with antibody P4 (Fig. 3D), a similar reaction pattern as described for non-enriched brainstem samples of case 1 (Fig. 2C, 2D). Cerebellum samples from case 2 reacted strongly with both antibodies, 6H4 and P4 (Fig. 3C, 3D). The intensity of the reaction of case 2 brain material with antibody P4 (Fig. 3D) was even stronger than with 6H4 (Fig. 3C) at similar milligram equivalent amounts. The sheep scrapie control sample showed a similar antibody reaction pattern as seen for case 2 (Fig. 3C, 3D).

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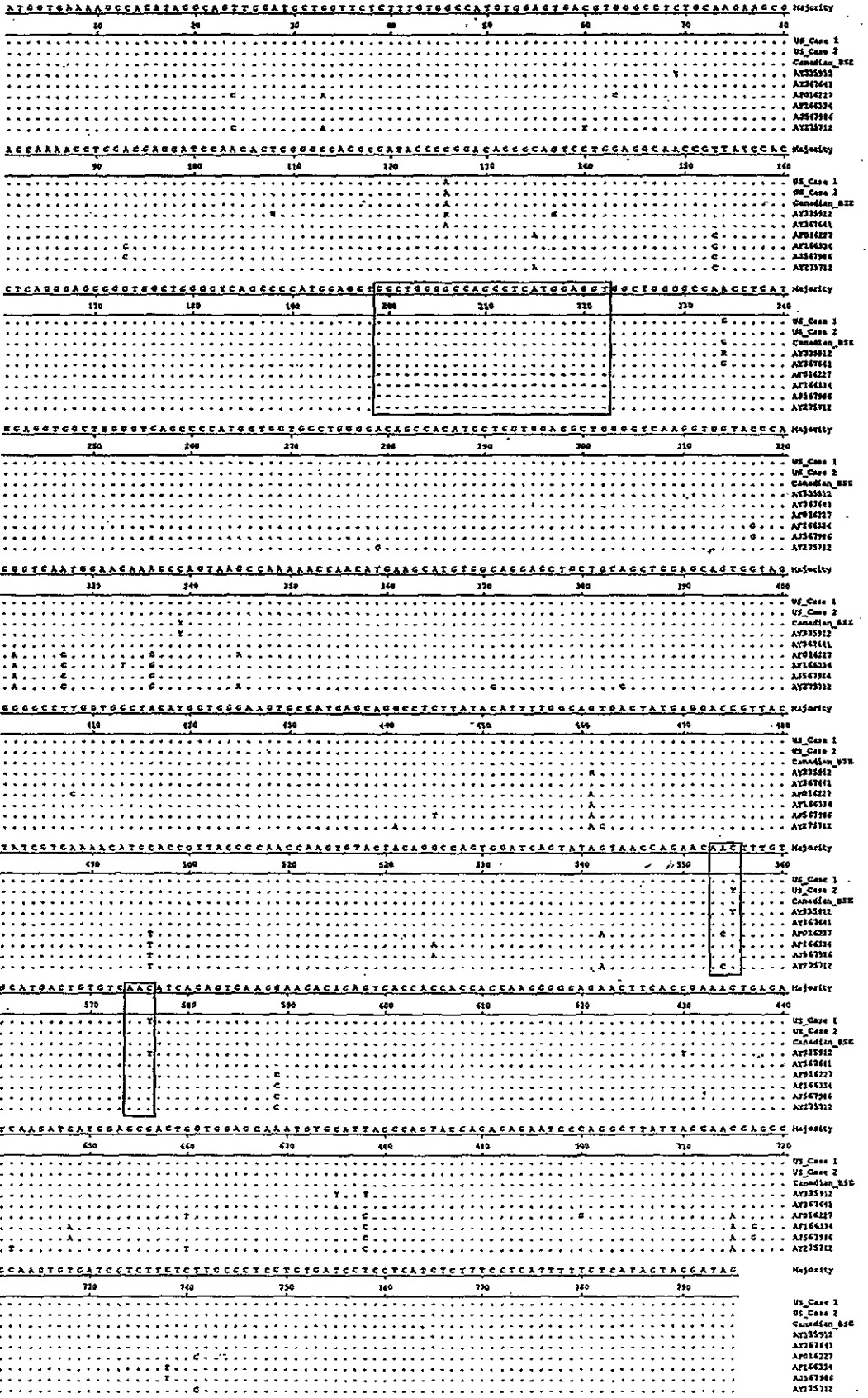
Figure 4. Alignment of bovine, ovine and cervid *Prnp* sequences including US BSE cases 1 and 2. A, Nucleotide sequences. Box enclosing nucleotides 199–222 (codons 67–74) represents an additional octapeptide-repeat region (total of 6 octapeptide repeats) but not in ovine and cervids (total of 5 octapeptide repeats). Box surrounding nucleotides 553–556 represents the synonymous polymorphism (AAC/AAT) located in the 3rd position of bovine codon 185 of US BSE Case 2. Box surrounding nucleotides 574–576 indicates the synonymous polymorphism at codon 192 (AAC/ AAT) of US BSE Case 1. Standard single letter codes are used for nucleotides. Y = C or T; R = A or G; K = G or T; W = A or T. B, Amino acid sequences. Standard IUPAC single letter codes are used for amino acids. Codon numbering refers to the most common 6-copy octapeptide repeat allele for *Bos Taurus*. The following bovine, cervid, and ovine GenBank entries were used for the alignments: US BSE Case 1, US BSE Case 2, Canadian May 2003 BSE case,[12] and GenBank accession numbers AY335912 (bovine: reports *Prnp* coding variation in a panel of 96 cattle chosen to represent most of the genetic diversity of the beef cattle breeds most commonly raised in North America), AY367641 (bovine), AF166334 (sheep), AJ567986 (sheep), AY275712 (white-tailed deer), and AF016227 (elk).



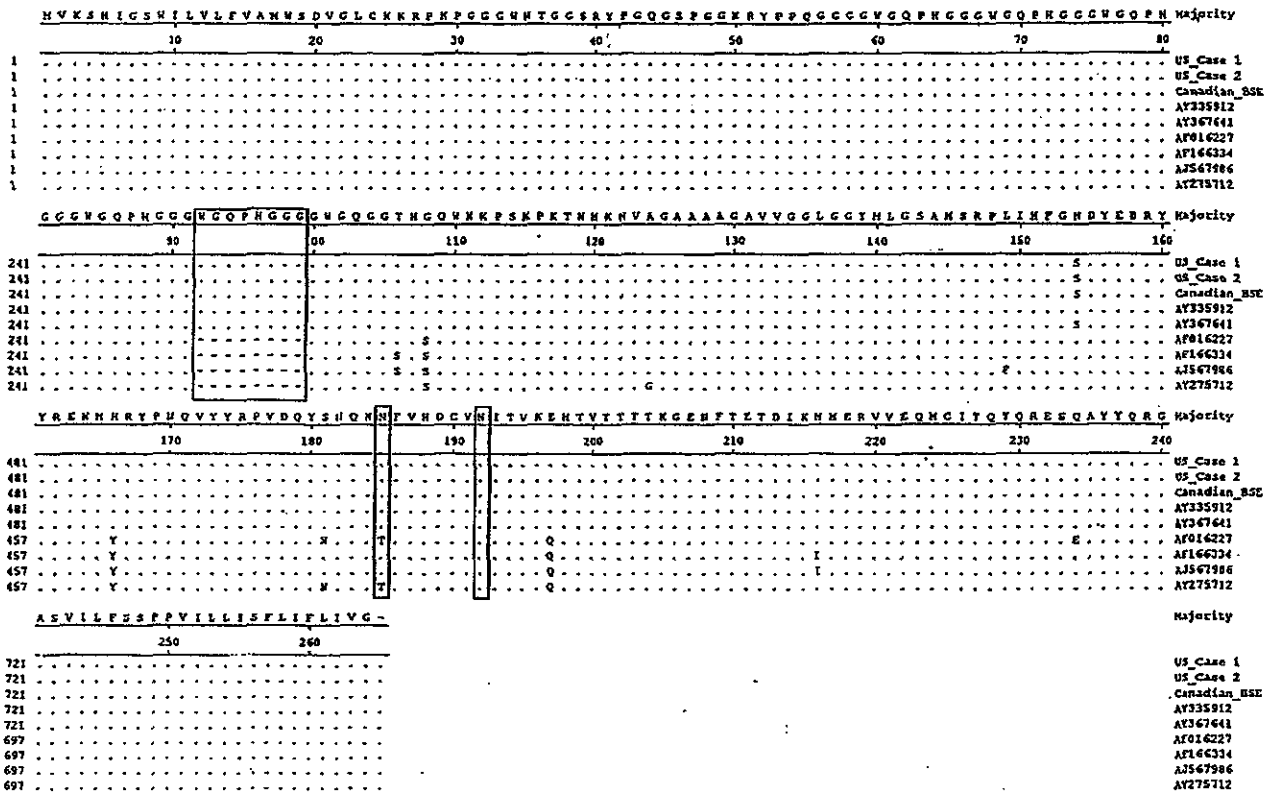


Figure 4. Continued.

Analysis of the Prnp

In order to confirm that the specimens from both cases were of cattle origin and to determine whether the might be associated with a spontaneous germline mutation, the full coding sequence from exon 3 of the *Prnp* was amplified and aligned with known PrP sequences from cattle, sheep, and cervids. DNA was isolated from brainstem material of both cases. As shown in Fig. 4A, the DNA sequence of both cases was identical to representative bovine *Prnp* sequences, but different from sheep and cervid sequences. The *Prnp* sequence of case 1 was found to be normal with a synonymous polymorphic position at codon 192 (T or C; no amino acid substitution). The animal had 6 copies of the octapeptide repeat region on both of their *Prnp* alleles. The *Prnp* gene of case 2 was also found to be normal with a synonymous polymorphism at codon 185 (T or C; no amino acid substitution), and both alleles contained the 6-copy octapeptide repeat region. The amino acid sequences of the 2 cases did not differ from each other and other bovine PrP protein sequences, however, there were differences when compared with the sheep and cervid sequences (Fig. 4B).

Discussion

The BSE epidemic in the United Kingdom was a food-borne disease in cattle, associated with feeding of meat and bone meal (MBM) that contained infected central nervous system tissue.[52] The cause of the original case or cases of bovine spongiform encephalopathy (BSE), however, remains an enigma. Sheep- or goat-derived scrapie-infected tissues included in the MBM rations fed to cattle or a previously undetected sporadic bovine TSE (maybe due to a germline mutation in the *Prnp* of affected cattle) have been considered as possible origins. Recently, another theory namely that BSE originated from a human TSE through animal feed containing imported mammalian raw materials contaminated with human remains from the Indian subcontinent has been brought forward.[12] Unlike CWD and scrapie, there is little evidence for either direct horizontal or vertical transmission of BSE among cattle.[2,53] Since the introduction of a ban on the use of ruminant proteins for ruminant feed in affected countries, the incidence rate of BSE in these countries has been steadily declining.[47]

This report presents the prion protein polypeptide profile and genotype from 2 cases of BSE diagnosed in the United States in 2003 and 2004. The obex area of the brainstem of case 1 was positive by a rapid BSE test, contained spongiform changes and had extensive deposition of the abnormal form of the prion protein, PrP^{Sc}, by IHC (Fig. 1A, 1B). Western blot analyses using brain material revealed a positive reaction using a 1-mg brain tissue equivalent (mg eq). The PrP^{Sc} polypeptide profile from BSE case 1 was characterized by 1) a lower molecular mass of the unglycosylated PrP^{Sc} polypeptide fragment compared to samples from sheep with scrapie and deer or elk with CWD, 2) good immunoreactivity with monoclonal antibody 6H4, and a lack of or weak staining with monoclonal antibody P4, and 3) a glycoform profile with a predominant proportion of the diglycosylated PrP^{BSE} isoform (see Fig. 2). Western blot comparison of the US BSE case 1 to the May 2003 Canadian and European BSE isolates revealed similar sized PrP^{Sc} polypeptide fragments (Fig. 2B). The 2003 Canadian BSE isolate from Alberta was reported to be a typical BSE isolate with similar molecular properties (including a lack of or weak staining with monoclonal antibody P4) to the PrP^{Sc} isolated from BSE cases in Switzerland (Fig. 2B) and the United Kingdom.[45] PrP^{Sc} isolated from elk with CWD had a higher molecular mass profile than did the corresponding PrP^{Sc} for the Canadian BSE case.[45] Similarly, PrP^{Sc} from mule deer and elk with CWD had a higher molecular mass profile than did the corresponding PrP^{Sc} for the US BSE case 1 (Fig. 2A, 2B).

United States BSE case 2 was born and raised in Texas, and represents the first native case of BSE in the United States. The brainstem of this animal reacted positive in the rapid BSE test used for BSE surveillance in the United States. The tissue of US BSE case 2 had been frozen before formalin fixation. Therefore, artifactual histopathological changes were present in the brainstem of this animal (Fig. 1C), however, unambiguous vacuolar changes diagnostic for BSE were not detected. PrP^{Sc} was detected in the brainstem by IHC (Fig. 1D). However, the staining intensity was less intense when compared with the signal found in the brainstem of case 1 (Fig. 1B). Moreover, the IHC staining pattern of case 2 was rather localized and not as diffuse as found with case 1. Interestingly, case 2 was only positive after formic acid treatment and extended antigen retrieval. Initially, case 2 was negative by IHC, even though the IHC positive control brainstem sections (from case 1) were strongly positive under the initial fixation and antigen retrieval conditions used. A positive Western blot reaction using antibody 6H4 was only seen after sample enrichment using the OIE SAF Immunoblot method and only 50% (5 out of 10) of brainstem or cerebellum samples were positive for the presence of PrP^{Sc} when tested at 20-mg brain tissue equivalent. This indicates that PrP^{Sc} was not uniformly distributed in the brainstem or cerebellum of case 2 and the PrP^{Sc} content per mg tissue equivalent was significantly lower (at least 20 times less) than found in brain material of case 1. Surprisingly, brain material from case 2 reacted strongly with antibody P4 (Fig. 3D). The intensity of the reaction of cerebellum and brainstem from case 2 with antibody P4 was even stronger than with 6H4 at similar mg tissue amounts (Fig. 3 C–D). A similar pattern was also seen with the sheep scrapie control sample (Fig. 3C–D). The unglycosylated and monoglycosylated isoforms of PrP^{Sc} from case 2 migrated higher than the respective isoforms of case 1 (Fig. 3B–C), indicating a difference in molecular mass between the 2 US BSE cases. The migration pattern of case 1 is typical for BSE (Fig. 2B), whereas the migration pattern of case 2 was reported as being unusual for BSE.[5] In addition, glycoform profile analysis of case 2 revealed a significant proportion of the diglycosylated isoform (approximately 61%), however, the percentage was lower than observed for the diglycosylated isoform of case 1 (approximately 72%). The monoglycosylated and unglycosylated isoforms of case 2 were more prominent (approximately 30% and 9%, respectively) than observed with the respective isoforms of case 1 (approximately 23% and 4%, respectively). The glycoform profile for case 1 is similar to that described for typical cases of BSE [5,28], whereas the profile found for case 2 is rather unusual.[5] The differences observed between case 1 and 2 were not caused by sampling of different regions of brain, since both, brainstem and cerebellum samples of case 1 and 2 showed the above described reaction patterns.

The *Prnp* gene of *Bos Taurus* contains multiple polymorphic sites: binary single nucleotide polymorphisms and an insertion-deletion polymorphism with 5, 6, or 7 repeats in the octapeptide-repeat region have been reported for exon 3.[13,25] The *Prnp* sequences of case 1 and 2 clearly fall within the range of cattle sequence diversity previously reported for cattle (Fig. 4A, 4B). Therefore, one etiological possibility for the disease condition of these animals, namely a germline mutation similar to one of the genetic forms of human prion disease can most likely be ruled out.[37] *Prnp* sequences from both US BSE cases contained 6 octapeptide-repeat regions on both alleles, a number found in cattle but not in either sheep or cervids. United States BSE case 1 had a polymorphic site at codon 192, a position previously reported to be variable in the bovine *Prnp* gene with

a genotype frequency of about 18% in the US beef cattle population.[25] Both, DNA amplified from fresh as well as paraffin-embedded tissues, showed the codon 192 polymorphism, indicating that these samples were derived from the same animal. United States BSE case 2 had a synonymous polymorphism at codon 185, reported to be variable with a genotype frequency of about 6% in the US beef cattle.[25] We conclude from these data that samples from both US BSE cases had normal, unremarkable cattle-like *Prnp* gene sequences. Polymorphisms in the sheep prion protein have been correlated with both variable incubation periods and degrees of resistance to scrapie.[19,20] This has not been the case for BSE in cattle.[29,34] However, noncoding regions of the *Prnp* gene locus of cattle (e.g., prion protein gene promoter polymorphisms) might have an influence on BSE susceptibility as reported recently.[43,44] These regions have not been analyzed in this study.

Unusual cases of BSE have been reported in the past 2 years by investigators from several countries. There have been 2 molecular types of unusual BSE isolates described in the literature: 1) a type with a lower molecular mass of the unglycosylated isoform (L-type) and 2) a type with higher molecular mass of the unglycosylated isoform (H-type). The L-type has been found in cattle in Italy,[11] Japan,[54] and Belgium.[15] In Italy, 2 cattle, older (11 and 15 years) than other bovines affected with BSE, showed an unusual molecular phenotype. Western blot analysis showed a PrP^{Sc} type with a predominance of the monoglycosylated isoform and the unglycosylated isoform fragment of lower molecular mass than usually seen with BSE.[11] The disorder was pathologically characterized by the presence of PrP^{Sc}-immunopositive amyloid plaques, as opposed to the lack of amyloid deposition in typical BSE cases, and by a different pattern of regional distribution and topology of brain PrP^{Sc} accumulation.[11] On the basis of the above features, the authors proposed to name the disease bovine amyloidotic spongiform encephalopathy, BASE.[11] A Japanese case was identified in an ELISA-positive specimen from a 23-month-old Holstein steer slaughtered in September 2003. The animal was reportedly healthy before slaughter and histology showed no spongiform changes and IHC revealed no signal of PrP^{Sc} accumulation typical for BSE. Western blot analysis of brainstem homogenate revealed a small amount of PrP^{Sc} with an electrophoretic profile different from that of typical BSE-associated PrP^{Sc} with 1) a lower content of the diglycosylated molecular isoform of PrP^{Sc}, 2) a faster migration of the unglycosylated isoform of PrP^{Sc}, and 3) less resistance against PK digestion when compared with typical BSE.[54] Another case involved a 64-month-old Belgian cow, whose obex sample was positive by ELISA, and the cow was reported healthy before slaughter. The histopathology of the obex, pons, and midbrain showed no spongiform changes and IHC of the brainstem revealed no signal of PrP^{Sc} accumulation. Western blot analysis of the obex region revealed a small amount of PrP^{Sc} with an electrophoretic profile of the unglycosylated isoform of PrP^{Sc} showing a lower migration pattern compared with that of a typical BSE case.[15]

So far, the H-type has been only described in cattle from France and Germany.[5,9] A distinct molecular phenotype was found in 1 German and 3 French BSE cattle following active surveillance of the disease at slaughterhouses or in rendering plants using rapid BSE tests. The 3 French cases did not have clinical signs suggestive of BSE during their life and the German and French animals were older cattle (8–15 years old). The unusual molecular phenotype of the German[9] and French[5] cases was characterized by 1) a higher molecular mass of the unglycosylated PrP^{Sc} isoform, 2) a strong labeling of all 3 PrP^{Sc} polypeptides with antibody P4, and 3) the French cases by a glycoform profile with a less prominent diglycosylated PrP^{Sc} isoform.[5] All of the H-type features were also observed with US BSE case 2 (Fig. 3).

Unusual cases of BSE are an unexpected finding since it was previously believed that BSE disease in cattle is caused by a single strain of infectious agent, which has been shown to be very consistent and uniform in appearance, even after transmission to other species.[7,28,46] The reports of unusual phenotypes of BSE in cattle suggest that different PrP^{Sc} phenotypes exist in cattle with BSE. There are several hypotheses which can explain these findings:[5] 1) there are manifestations of the BSE agent with different molecular features in cattle. It is known that sequence differences in the *Prnp* gene give rise to variants in electrophoretic profiles of PrP^{Sc}, as shown in cases of human CJD,[10] however, comparison of the *Prnp* alleles available from cattle with unusual BSE phenotypes (including US BSE case 2) and the general cattle population have shown normal cattle *Prnp* sequences; 2) cattle may have been infected by another source of infectious agent (e.g., scrapie or CWD). However, a survey of brain material derived from 262 high-risk, adult cattle in CWD-endemic areas in Colorado for the presence of changes indicative of a TSE infection was negative.[22] Interestingly, experimental infection of cattle with either US sheep scrapie isolates[14] or CWD sources[23,24] led to a cattle disease with clinicopathological features different from typical BSE in cattle; 3) a rare sporadic form of TSE disease could exist in cattle as described for human TSEs.[16] Further studies are needed to

determine the frequency and origin of such novel BSE phenotypes. Critical studies would elucidate the transmissibility of unusual BSE cases to cattle or other mammalian hosts; transmission has not been reported in the literature so far.

It is concluded from the studies reported here that 1) the PrP^{Sc} profile from the first US BSE case showed similar molecular properties to the typical PrP^{Sc} pattern described for the May 2003 Canadian and European BSE isolates,[4,5] and 2) the PrP^{Sc} profile from the second US BSE case showed unusual molecular properties similar to atypical high molecular weight BSE cases reported in France and Germany.[5,9] Both cases were identified by the USDA surveillance program in place and the carcasses did not enter the human or animal food chain. IHC staining in brainstem sections for an unusual high molecular BSE case is described here for the first time. A germline mutation as an etiological possibility for the disease conditions of both cases can be most likely ruled out. Future work will address the question whether brain material from both US BSE cases are infectious in cattle after intracerebral and oral inoculation.

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Sources and manufacturers

a. Platelia/TeSeE™ ELISA BSE test, Bio-Rad, Hercules, CA.
b. Fisher Superfrost Plus, Fisher Scientific, Hampton, NH.
c. Dako, Carpinteria, CA.
d. Biocare, Walnut Creek, CA.
e. Ventana Medical Systems Inc., Tucson, AZ.
f. VMRD, Pullman, WA.
g. Non Ultra Dish Liquid, Original Scent, Dawn. Procter & Gamble, Cincinnati, OH.
h. Prionics, Schlieren, Switzerland.
i. Fisher Scientific, Pittsburgh, PA.
j. Invitrogen, Carlsbad, CA.
k. Immobilon-P, Amersham Biosciences, Piscataway, NJ.
l. R-Biopharm Inc, Marshall, MI.
m. Amersham Biosciences, Piscataway, NJ.
n. Novagen, Madison, WI.
o. Sigma, St. Louis, MO.
p. Beckman Coulter Inc, Fullerton, CA.
q. USB, Cleveland, OH.
r. Roche, Indianapolis, IN.
s. Kodak, St. Louis, MO.
t. Qiagen, Valencia, CA.
u. Amresco, Solon, OH.
v. Strategene, La Jolla, CA.
w. Q-BIOgene, Ivine, CA.
x. Big Dye Teminator, Applied Biosystems, Foster City, CA.

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 5月6 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			研究報告の公表状況	Transmission and adaptation of chronic wasting disease to hamsters and transgenic mice: evidence for strains. Raymond, G. J. et al, J. Virol., 81, 4305 -4314 (2007).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
慢性消耗性疾患（CWD）の新規げっ歯類モデルを作製するために，初めて無細胞プリオン蛋白質（PrP）変換測定法を用いて種々のハムスター及びマウスの動物種の感受性を評価した。本測定法では，3 種シカ（ヘラジカ，オジロジカ及びミュールジカ）から得られたプロテイナーゼ K 耐性 PrP による，げっ歯類動物の正常なプロテイナーゼ K 感受性 PrP から耐性 PrP への変換反応を評価した。その結果，一部のげっ歯類のみが感受性を有することが示唆された。これらの結果は CWD 罹患シカの脳組織をげっ歯類の脳内に接種した際に in vivo でも確認された。検討したげっ歯類動物のうち，遺伝子組み換えマウスがシリアンゴールデンハムスターのプリオン蛋白を発現したのに加えて，アルメニアン，チャイニーズ，シベリアン，シリアンゴールデンは限定的ではあるが感受性を示した。状態が悪化した少数の動物は，長期の潜伏期間の後に疾患の臨床徴候を発現した。ジャンガリアンハムスター及び野生型マウスは罹患しないままであった。しかし，シリアンゴールデンハムスターにおける CWD 分離株の連続継代によって，明らかな神経病理学的特徴を示し，臨床症状を呈するまでの平均潜伏期間が異なる 2 つのプリオン分離株を特定した。また，当該動物種では接種を受けた動物の全てが第 2 及び第 3 継代の後，疾患を発現した。これらの結果から，CWD は一部のげっ歯類の動物種に伝播し，様々な分離株が存在する可能性があることが示された。	BYL-2007-0281

報告企業の意見	今後の対応
この報告はげっ歯類モデルの脳内伝播で特異的な神経病理学的疾患を引き起こす CWD の様々なプリオン株の存在を強く示唆するものである。これらの CWD プリオン株は異種宿主への連続継代においてより発現率が高くなる傾向にある。この研究報告は CWD でも種の壁を崩壊する可能性があることを示唆している。	これまでに，種の壁が崩壊したという報告例はないため，現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

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Transmission and Adaptation of Chronic Wasting Disease to Hamsters and Transgenic Mice: Evidence for Strains▽

Gregory J. Raymond,[1] Lynne D. Raymond,[1] Kimberly D. Meade-White,[1] Andrew G. Hughson,[1] Cynthia Favara,[1] Donald Gardner,[2] Elizabeth S. Williams,[3]† Michael W. Miller,[4] Richard E. Race,[1]* and Byron Caughey[1]*

Laboratory of Persistent Viral Diseases[1] and Rocky Mountain Veterinary Branch,[2] NIAID, NIH, Rocky Mountain Laboratories, Hamilton, Montana 59840; Department of Veterinary Sciences, University of Wyoming, Laramie, Wyoming 82070[3]; and Colorado Division of Wildlife, Wildlife Research Center, Fort Collins, Colorado 80526-2097[4]

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In vitro screening using the cell-free prion protein conversion system indicated that certain rodents may be susceptible to chronic wasting disease (CWD). Therefore, CWD isolates from mule deer, white-tailed deer, and elk were inoculated intracerebrally into various rodent species to assess the rodents' susceptibility and to develop new rodent models of CWD. The species inoculated were Syrian golden, Djungarian, Chinese, Siberian, and Armenian hamsters, transgenic mice expressing the Syrian golden hamster prion protein, and RML Swiss and C57BL10 wild-type mice. The transgenic mice and the Syrian golden, Chinese, Siberian, and Armenian hamsters had limited susceptibility to certain of the CWD inocula, as evidenced by incomplete attack rates and long incubation periods. For serial passages of CWD isolates in Syrian golden hamsters, incubation periods rapidly stabilized, with isolates having either short (85 to 89 days) or long (408 to 544 days) mean incubation periods and distinct neuropathological patterns. In contrast, wild-type mouse strains and Djungarian hamsters were not susceptible to CWD. These results show that CWD can be transmitted and adapted to some species of rodents and suggest that the cervid-derived CWD inocula may have contained or diverged into at least two distinct transmissible spongiform encephalopathy strains.

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy (TSE), or prion disease, that affects mule deer (*Odocoileus hemionus*), black-tailed deer (subspecies of *Odocoileus hemionus*), white-tailed deer (*Odocoileus virginianus*), Rocky Mountain elk (*Cervus elaphus nelsoni*), and moose (*Alces alces*) (17). Other TSE diseases include scrapie in sheep and goats, transmissible mink encephalopathy (TME), bovine spongiform encephalopathy (BSE), and Creutzfeldt-Jakob disease in humans. Some TSE diseases, such as scrapie, BSE, and Creutzfeldt-Jakob disease, have been experimentally transmitted and adapted to rodents, yielding experimental animal models that have proved useful in the study of TSE diseases (4, 10, 13). Recently, CWD was transmitted to mice transgenic for cervid PrP (16a, 24). Although CWD has been transmitted to ferrets, and then from ferrets to Syrian golden (Sg) hamsters (2), there have been no reports of direct transmissions of CWD to any hamster species or wild-type mice (25).

An important and measurable occurrence in TSE diseases is the conversion of the normal host proteinase K (PK)-sensitive prion protein (PrP-sen) to an abnormal, disease-associated isoform that is characteristically PK resistant (PrP-res). One in vitro method that has been used to assess the potential interspecies transmissibility of TSE agents is a cell-free conversion (CFC) reaction in which PrP-res from one species is tested for its relative efficiency in converting PrP-sen of other species to PrP-res (20, 23). Such CFC reactions were found in the present study to predict the susceptibility of hamsters to CWD. Accordingly, CWD isolates were inoculated into various species of rodents, and some species proved to be modestly susceptible. The resulting rodent-adapted CWD models could be useful in comparative studies of TSE strains in vivo as well as for testing potential anti-TSE therapeutic agents.

MATERIALS AND METHODS

PrP-sen ^{35}S labeling and purification. The generation and detailed analyses of the cervid and human PrP-sen molecules have been described previously (6, 20). The PrP polymorphic types used in this study were as follows: elk e-GLSE and e-GMSE (PrP amino acid residues 96, 132, 138, and 226 are designated); md/wd-GMNQ and md/wd-GMSQ, which are identical in mule deer and white-tailed deer; wd-SMSQ, found only in white-tailed deer; and human, hu-M and hu-V (residue 129 is designated). The Sg hamster PrP-sen construct lacks a glycosylphosphatidylinositol (GPI) anchor (15). The ^{35}S–PrP-sen molecules of the various species were immunopurified from various cultured cell lines labeled metabolically with [^{35}S]methionine (EasyTag; Perkin-Elmer) (8), and each cell line used expressed one of the PrP-sen types. In order to simplify analysis of PrP conversion products, the ^{35}S–PrP-sen molecules were radiolabeled in the presence of 10 μg/ml tunicamycin (Roche), an inhibitor of glycosylation. R521 antibody (Ab) (20) was used for immunoprecipitation of the various cervid ^{35}S–PrP-sen molecules, and 3F4 monoclonal Ab (11) was used for the human and hamster PrP-sen molecules.

PrP-res purification. Hamster PrP-res (ha263K) was purified from the brains of 263K-affected Sg hamsters (21). PrP-res isolates from brainstems of CWD-affected elk (eCWD), mule deer (mdCWD), and white-tailed deer (wdCWD) were the same as those used in a previous study (20) and were purified using the same method as that for ha263K.

CFC reactions. CFC reaction methods have been described previously (20, 22) and are summarized here. For each CFC reaction, 250 ng of each of the purified, nonradiolabeled PrP-res molecules isolated from brain pools (20) was incubated with 20,000 to 30,000 cpm of each of the immunopurified ^{35}S–PrP-sen molecules. PrP-res was pretreated with 2.0 to 2.5 M guanidine hydrochloride at 37°C for 1 h,

* Corresponding author. Mailing address: Rocky Mountain Labs, 903 S. 4th St., Hamilton, MT 59840. Phone for Byron Caughey: (406) 363-9264. Fax: (406) 363-9286. E-mail: bcaughey@nih.gov. Phone for Richard Race: (406) 363-9358. Fax: (406) 363-9286. E-mail: rrace@nih.gov.
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added to the ^{35}S–PrP-sen with final concentrations of 1 M guanidine-HCl, 50 mM sodium citrate, pH 6.0, 5 mM cetylpyridinium chloride, and 1.25% Sarkosyl, mixed, and incubated for an additional 72 h at 37°C. One-eleventh of each reaction mix was used for the mock (no-PK) digestion control, and the remainder was treated with 20 μg/ml PK for 1 h at 37°C. One microliter of 0.1 M Pefabloc (Roche) was added to each sample, and the samples were methanol precipitated, pelleted, boiled in polyacrylamide gel electrophoresis (PAGE) loading buffer, and run in precast 16% sodium dodecyl sulfate-PAGE (SDS-PAGE) gels (Invitrogen). A Storm phosphorimager (GE Healthcare) was used for detection, and ImageQuant software was used to quantitate radioactive PrP bands. The conversion efficiency of each reaction was the percentage of input ^{35}S–PrP-sen (determined from the no-PK aliquot) that was converted to 16- to 18-kDa PK-resistant ^{35}S-PrP bands (determined from the PK-treated aliquot).

Animals. Sg hamsters (*Mesocricetus auratus*) were purchased from Harlan Sprague Dawley, Inc. Armenian (*Cricetulus migratorious*), Chinese (*Cricetulus griseus*), Djungarian (*Phodopus campbelli*), and Siberian (*Phodopus sungorus*) hamsters and RML Swiss mice and Tg (haPrP) mice ("Sg hamsterized;" also called Tg7-haPrP/moPrP$^{-/-}$ mice) (18) were bred at NIAID/Rocky Mountain Laboratories (RML). C57BL10 mice were purchased from Jackson Laboratory. Protocols for using animals in these studies were reviewed and approved by the NIAID/RML Animal Care and Use Committee and complied with relevant NIH guidelines. Animals were housed at NIAID/RML facilities accredited by AAALAC International.

CWD primary inocula. New supplies and aseptic technique were used for the following preparations to minimize the potential for contamination from any other TSE source. In addition, inoculations for these experiments were done separately from any other TSE work. For each of the individual brain inocula used (see Fig. 3A), the brainstems of a CWD-affected elk, mule deer, and white-tailed deer were removed. The brainstems were confirmed to be CWD positive by histology and immunoblot analysis of PrP^{CWD}, and homogenates were inoculated into Sg hamsters and RML and Tg (haPrP) mice. Each of the CWD brain pools used (see Fig. 3B) has been described previously and contains heterogeneous genotypes (20). Brain pool-derived homogenates were inoculated into Sg, Djungarian, Chinese, and Armenian hamsters and C57BL10 mice. For each of the inocula, 10% brain homogenates in Dulbecco's phosphate-buffered saline (DPBS; Invitrogen) were made using a separate, new Dounce (Wheaton) homogenizer. Suspensions were sonicated for 5 min at maximum power (Heat Systems-Ultrasonics), diluted to 1% in DPBS, and inoculated intracerebrally (i.c.; 0.05 ml per animal).

Second- and third-passage inocula. For the second passage, each brain from rodents inoculated with the individual animal primary inocula and suspected of being TSE positive by progressive signs of neurological disease was excised using new tools and divided sagittally. Half of each brain was used for immunohistochemical analysis (described below). A 20% homogenate was made from the other half in DPBS, using new disposable plastic pestles and microtubes (Kontes). A portion of this homogenate was used for PrP-res detection on immunoblots (see below), and another portion was diluted to 1%, with 0.05 ml inoculated i.c. into each animal as outlined in Fig. 3. The CWD brain pools from elk, mule deer, and white-tailed deer were passaged only once.

Immunoblot analysis of PrP-res from brain tissue. Aliquots (0.1 ml) of 2% Triton X-100, 2% sodium deoxycholate, 0.2 M Tris-HCl, pH 8.3 (at 22°C), 0.3 M NaCl, and 0.01 M EDTA were added to 0.1-ml aliquots of the 20% brain homogenates described above. After incubation for 60 min at 22°C, the samples were placed on ice and sonicated in a cup horn at maximum power for 1 min. After centrifugation at 2,500 × *g* for 10 min at 4°C, the tubes were inverted three times to resuspend the softer portions of the pelleted material. The resulting supernatant suspensions, including all but the hardest parts of the pellets, were removed, and aliquots were saved at −20°C. A 15-μl aliquot of each suspension was digested by adding PK to 50 μg/ml and was incubated at 37°C for 60 min. One microliter of 0.1 M Pefabloc (Roche) was added, and the sample was held on ice for 5 min, followed by the addition of 25 μl of 2× PAGE loading buffer with 50 mM dithiothreitol and boiling for 5 min. Ten microliters of 0.25 M iodoacetamide (Sigma) was then added and incubated at 37°C for 10 min. A 10-μl aliquot of each sample was subjected to electrophoresis on 10% NuPAGE bis-Tris gels, using morpholineethanesulfonic acid (MES) running buffer (Invitrogen). Proteins were transferred to Immobilon-P (Millipore) membranes by semidry electroblotting. Membranes were immunostained using the following primary Abs (as described in references 20 and 22): monoclonal Ab 3F4 (11), rabbit antiserum 505 (against sheep peptide residues 100 to 111 [20]; generously provided by J. Langeveld, CIDC-Lelystad, The Netherlands), and rabbit antiserum R30 (against Sg hamster PrP residues 89 to 103) (9). The immunoblot was incubated with either alkaline phosphatase-conjugated anti-mouse or anti-rabbit immunoglobulin G (IgG; Zymed) secondary Ab, developed using AttoPhos (Promega) substrate, and scanned using a Storm fluorescence detection instrument (GE Healthcare).

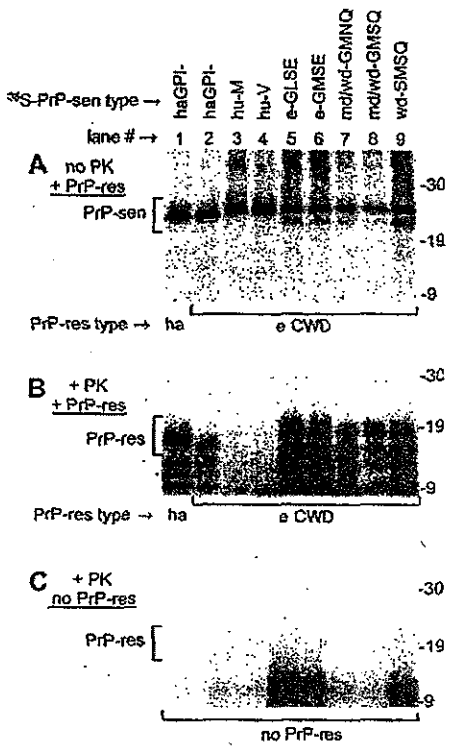

FIG. 1. CFC reactions of various species' PrP-sen molecules induced by PrP-res. PrP-res isolated from either Sg hamsters (ha; lane 1 of each panel) or CWD-affected elk (e CWD; lanes 2 to 9) was incubated with various immunopurified ^{35}S–PrP-sen molecules, identified at the tops of the lanes. (A) Aliquots (1/11) of each reaction mix that were not PK treated. The PrP-sen bands (bracketed; 22 to 26 kDa) were not glycosylated, as explained in Results. (B) Remaining portion of each reaction mix, treated with PK, resulting in the radiolabeled PrP-sen molecules being converted to PK-resistant PrP bands (PrP-res bracket; 16 to 18 kDa). (C) Parallel set of PK-digested reaction mixes incubated without PrP-res. The following PrP-sen molecules (descriptions are given in reference 20) were used in the conversion reactions: cervid PrP polymorphic types present in elk (e-GLSE and e-GMSE; lanes 5 and 6), PrP types present in mule deer and white-tailed deer (md/wd-GMNQ and md/wd-GMSQ; lanes 7 and 8) or white-tailed deer only (wd-SMSQ; lane 9), human Met/Met (hu-M; lane 3) and human Val/Val (hu-V; lane 4) PrP (amino acid residue 129 is given), and Sg hamster PrP lacking the GPI anchor (lanes 1 and 2; haGPI−). The md/wd GMNQ (lane 7) has recently been shown to be only the predicted translation product of an unexpressed pseudogene in deer (6). All data except those using hamster PrP molecules were previously published (20) and are shown here only to compare interspecies conversion efficiencies. The hamster conversion reactions were done at the same time as the other conversion reactions, using identical PrP-res isolates. The migration of molecular mass standards, in kilodaltons, is shown to the right of each panel.

Immunohistochemical analyses. Brains were excised from PBS-perfused animals and divided sagittally. One-half was fixed in 3.7% phosphate-buffered formaldehyde for 3 to 5 days prior to routine dehydration and paraffin embedded. Sections were cut 4 to 6 μm thick and placed onto charged microscope slides. The remaining half was not fixed and was used for the biochemical analyses and serial passages described above. A Ventana NesES automated stainer was used for immunohistochemical staining of the sections. For PrP analysis, slides were deparaffinized, rehydrated with 0.1 mM citrate buffer, pH 6.0, and pretreated for 20 min at 120°C, using a decloaking chamber (Biocare). Using standard avidin-biotin technique (12), 3F4 was diluted 1:50, followed by biotinylated horse

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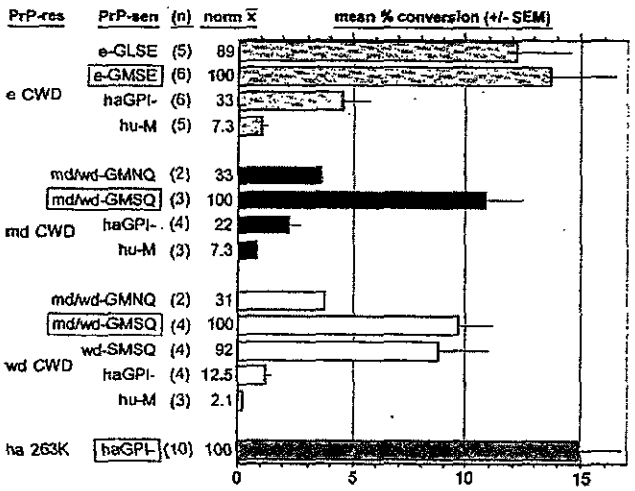

FIG. 2. Relative efficiencies of conversion reactions, examples of which are shown in Fig. 1. The efficiency for each conversion reaction was determined by quantitation of the amount of input radiolabeled ^{35}S–PrP-sen converted to ^{35}S–PrP-res bands, as described in Materials and Methods. Efficiencies are expressed as the mean percent conversion of ^{35}S–PrP-sen to ^{35}S–PrP-res for 2 to 10 replicate experiments (n), with standard errors of the means (SEM) shown by the error bars. For each PrP-res species, the mean efficiency was normalized (norm x) to the mean conversion efficiency of the homologous (boxed) PrP-sen. All data except those using hamster PrP molecules were previously published (20) and are shown here for comparison.

anti-mouse IgG at 1:250 (Vector Laboratories), Biogenex SS streptavidin (Biogenex), and amino carbazole as the substrate (Ventana). For glial fibrillary acidic protein (GFAP), there was no pretreatment, and the standard avidin-biotin technique was used with anti-GFAP at 1:1,000 (DAKO), biotinylated goat anti-rabbit IgG at 1:250 (Vector), and amino carbazole. Images were magnified at 40× and were captured on an Olympus BX51 light microscope, using MicroSuite software. For the images of whole brain sections, stained microscope slides were scanned using an Epson Expression 1640XL scanner at 1,400 dpi, and the images were processed using Adobe Photoshop software.

RESULTS

CFC reactions. To test initially for the likelihood that hamsters might be susceptible to CWD, interspecies CFC reactions were done. Purified PrP-res from CWD-affected cervid brain tissue (PrP^{CWD}) was incubated with unglycosylated and immunopurified ^{35}S-labeled PrP-sen from hamsters, humans, and cervids and then digested with PK to detect newly formed ^{35}S–PrP-res (Fig. 1). Although the hamster PrP-sen (Fig. 1A, lanes 1 and 2) used in these reactions lacked the GPI anchor, the other PrP-sen molecules (Fig. 1A, lanes 3 to 9) used did not. Previous studies have shown that this difference does not significantly affect conversion efficiency, at least for hamster PrP (15, 16). Furthermore, glycosylation does not significantly affect conversion efficiencies under these conditions (22).

PK-resistant ^{35}S-PrP bands (Fig. 1B, PrP-res bracket) that were 6 to 8 kDa smaller than the ^{35}S–PrP-sen precursors (Fig. 1A, PrP-sen bracket) were of primary interest because they reflect the 6- to 8-kDa amino-terminal truncation that is observed with the PK digestion of PrP-res isolated from the brains of the TSE-affected species used for these studies (20). These CFC reactions show that elk PrP^{CWD} (eCWD) induced the conversion of cervid, human, and hamster ^{35}S–PrP-sen

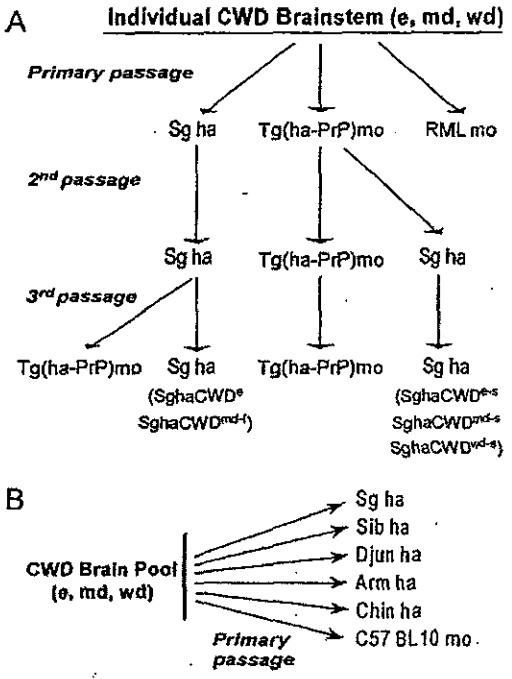

FIG. 3. Passage history of CWD inocula in rodent species. Primary inocula used were brain homogenates of each species (e, elk; md, mule deer; and wd, white-tailed deer) from either individual CWD-positive animals (A) or brain pools from CWD-affected animals of each species (B). The individual brains were passaged serially three times in the rodent species indicated, whereas the pooled brain samples were passaged only one time in the various species shown. Since there were no positive RML mice from the primary passage of the individual positive cervid brains, no additional passes were done. ha, hamster; Sib, Siberian; Djun, Djungarian; Arm, Armenian; Chin, Chinese; mo, mouse.

molecules to ^{35}S–PrP-res (16- to 18-kDa PrP-res [brackets in Fig. 1B]). Cervid and human ^{35}S–PrP-sen molecules were the most and least efficiently converted, respectively (20). Similar results were observed for CFC reactions done using mule deer (mdCWD) and white-tailed deer (wdCWD) PrP^{CWD} (Fig. 2). The hamster ^{35}S–PrP-sen was converted with intermediate efficiency by all PrP^{CWD} isolates (e-, md-, and wdCWD) and with a much higher efficiency by hamster PrP-res (ha 263K) (Fig. 2). For unknown reasons, the conversion products from PrP^{CWD} reaction mixes incubated with hamster ^{35}S–PrP-sen migrated slightly faster by SDS-PAGE than those induced by hamster PrP-res (Fig. 1B, lanes 1 and 2). In the absence of PrP-res, 16- to 18-kDa PK-resistant ^{35}S-PrP bands were not observed (Fig. 1C). The observation that the PrP^{CWD} preparations converted hamster ^{35}S–PrP-sen suggested that hamsters might be susceptible to CWD infection.

Passages of CWD isolates into Sg hamsters. Based on the CFC results, we attempted to transmit different CWD isolates to various species of hamsters as diagrammed in Fig. 3. Immunoblotting showed that PK-treated aliquots of the primary inocula from individual cervid animals (Fig. 4A, lanes e, md, and wd) and brain pools (20) contained PrP^{CWD}, as evidenced by immunoreactivity with antiserum 505, which detects PrP molecules from all of these species (20) (Fig. 4A, left panel). The same samples were not immunoreactive with monoclonal Ab 3F4, which detects PK-treated Sg hamster 263K PrP-res

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TABLE 1. Serial passage details of individual CWD-positive cervid animal brain homogenates inoculated into Sg hamsters

CWD inoculum	Primary passage				Second passage					Third Passage		
	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested[c]	Incubation time (dpi) of blot-positive samples[f]	Donor animal inoculum (dpi)[e]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time of animals with TSE signs (mean dpi ± SD)	Donor animal inoculum[e]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	Incubation time of animals with TSE signs (mean dpi ± SD)
e	Sg ha	0/7	2/7	381, 668	e 1 (381)	Sg ha	8/8	6/6	427 ± 53	e 1a	Sg ha	6/6	463 ± 21
										e 1b	Sg ha	4/4	423 ± 28
										e 1b	Tg (haPrP) mo	3/3	376 ± 8
md	Sg ha	2/7	4/7	172,* 274, 326,* 393 (291 ± 93)	md 1 (172)	Sg ha	8/8	2/2	85 ± 0	md 1a	Sg ha	6/6	89 ± 0
					md 2 (326)	Sg ha	8/8	2/2	85 ± 0	md 2a	Sg ha	6/6	89 ± 0
wd	Sg ha	0/8	0/8	None[d]	Not done					Not done			

[a] Sg ha, Sg hamster; Tg (haPrP) mo, Tg mouse with mouse PrP gene knocked out and Sg hamster PrP gene inserted (18).
[b] For each group, 12 animals were inoculated for the first passage, 8 animals were inoculated for the second passage, and six Sg hamsters or four Tg (haPrP) mice were inoculated for the third passage. Animals lost due to intercurrent deaths were not included in these data. Intercurrent deaths are defined as animals that died without neurological signs prior to the date that the first animal in the group was determined to be TSE positive by either neurological signs or brain PrP-res by immunoblot analysis.
[c] During the first passage, all animals with neurological signs were PrP-res positive, but not all brain PrP-res immunoblot-positive animals had neurological signs.
[d] The animals in this group did not have neurologic signs and were not positive for brain PrP-res during their life span (range, 351 to 553 dpi; mean, 451 ± 62 dpi).
[e] The code designates the individual Tg (haPrP) mouse or Sg hamster that was used as a source of inoculum in the passage specified. For example, "e 1" designates an individual TSE-positive Sg hamster that had been inoculated with eCWD and was used as the source of inoculum for a second passage; "e 1a" designates an individual Sg hamster from the second passage that had been inoculated from the "e1" Sg hamster.
[f] The days to death for the individual animals displaying neurological signs consistent with TSE disease are indicated by asterisks. Brain homogenates from each of these were inoculated into groups of eight Sg hamsters for the second passage.

FIG. 4. Analysis of PrP-res from sequential passages of CWD inocula in Sg hamsters and Tg (haPrP) mice. (A) Fluorescent immunoblots of SDS-PAGE gels show PK-resistant PrP in CWD primary inocula from individual elk (e), mule deer (md), and white-tailed deer (wd). Equal aliquots of the primary inocula were immunoblotted and analyzed with either 505 antiserum for the left blot or 3F4 Ab for the right blot. The 3F4 Ab detected 263K PrP-res from Sg hamster brain (ha), but it did not detect the PrP^{CWD} in any of the primary inocula. (B and C) Immunoblots of representative examples of first and second serial passages, respectively, into either Sg hamsters or Tg (haPrP) mice, analyzed using 3F4. The sources indicated in panels B and C identify the primary CWD passage inocula. The migration of molecular mass standards, in kilodaltons, is shown to the right of each panel.

(Fig. 4A, right panel). Thus, the 3F4 Ab was used to detect newly formed PrP-res in the host and to discriminate it from the primary inocula. Since 3F4 does not detect mouse or Chinese hamster PrP, PrP-res from these species was detected on immunoblots using R30 (data not shown), which, like 3F4, does not react with cervid PrP.

Upon primary passage of the various individual cervid inocula into Sg hamsters, only two hamsters inoculated with mule deer CWD showed clinical signs of TSE disease during an observation period of up to 2 years (Table 1, primary passage column, 172 and 326 days to death postinoculation [dpi]). Although they did not show evident clinical signs of TSE disease, two additional Sg hamsters for each of the md- and eCWD inoculations showed disease-associated PrP-res-positive immunoblots, indicating subclinical infections. None of the

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Sg hamsters inoculated with wdCWD showed any signs of neurological disease or tested positive for PrP-res on immunoblots.

When the TSE-positive Sg hamsters from the primary passage of eCWD were passaged a second and then a third time, all recipients showed clinical signs after prolonged mean incubation periods of ≥423 dpi (Table 1, second- and third-passage columns), and all of the brains tested were PrP-res positive on immunoblots. Passage of an inoculum from one of the second-passage eCWD-inoculated positive hamsters into Tg (haPrP) mice gave a slightly shorter mean incubation period (Table 1, third-passage column). Second and third passages of mdCWD into Sg hamsters gave much shorter incubation periods, averaging 85 to 89 dpi, suggesting that the mule deer-derived CWD isolate was a different and much faster (f) isolate (SghaCWD$^{md\text{-}f}$) than that obtained from the eCWD inoculum (SghaCWD^{e}). The TSE neurological signs of infection with this fast isolate differed from those for the SghaCWD^{e} isolate, with affected animals presenting initially with a waddling gait, head bobbing, and unkempt appearance that developed into severe ataxia to the point of repeated falling and loss of the righting reflex. Approximately 2 to 3 weeks after the first neurological signs were observed, these animals became recumbent and were euthanized. In contrast, SghaCWD^{e}-infected animals presented with a more subtle neurologic disorder that progressed at a much lower rate. After a period of 1.5 to 3 months, the disease typically progressed to hind leg paralysis, increased ataxia, tremors, and eventually, wasting.

Passages into "Sg-hamsterized" Tg (haPrP) mice. Tg (haPrP) mice overexpressing Sg hamster PrP on a mouse PrP null background have shorter incubation periods than do Sg hamsters when inoculated with 263K hamster-adapted scrapie (18). Based on this observation, we suspected that Tg (haPrP) mice may have a more rapid disease response to CWD infection than that of Sg hamsters. Therefore, the same cervid inocula used for the Sg hamsters were used for the Tg (haPrP) mice (Fig. 3A, primary passage). After inoculation with brain homogenates from each of the CWD-affected cervid species, approximately one-third of the Tg (haPrP) mice showed clinical signs of TSE disease after extended mean incubation periods ranging from 585 to 668 dpi (Table 2, primary passage column), and a majority (62 to 88%) of these mice were positive for brain PrP-res by immunoblot analysis (examples are shown in Fig. 4B). Second and third serial passages into Tg (haPrP) mice caused clinical disease in all of the recipients and reductions in average incubation periods of the various groups to 185 to 282 dpi. Sg hamsters receiving second and third passages from clinically affected Tg (haPrP) mice were much slower (s) to develop disease (408 to 544 dpi) with elk (SghaCWD$^{e\text{-}s}$), mule deer (SghaCWD$^{md\text{-}s}$), or white-tailed deer (SghaCWD$^{wd\text{-}s}$)-derived CWD isolates than when Tg (haPrP) mice were inoculated with the same material. The longer incubation periods observed for the Sg hamsters compared to those for the Tg (haPrP) mice remained stable upon additional passages (Table 2, second- and third-passage columns). These hamsters displayed neurological signs and disease courses that were similar, if not identical, to those seen with the SghaCWD^{e} isolate described above. The brains of all mice and hamsters analyzed for PrP-res on immunoblots were positive (examples are given in Fig. 4B and C). The clinical presentation of all affected Tg (haPrP) mice was similar to that for the SghaCWD^{e} isolate, with subtle neurological disturbances and a prolonged disease course lasting at least 1 to 2 months. The Tg (haPrP) mice also exhibited kyphosis, a tiptoed gait, hind leg clasp when suspended by the tail, and eventually hind leg paralysis in most of the animals. This disease course contrasted with that seen with 263K scrapie-inoculated Tg (haPrP) mice, in which subtle neurological symptoms are followed rapidly by death, within 2 to 4 days (18).

Immunohistological analyses of different isolates. Immunohistological analyses of brain sections from Sg hamsters clinically affected with the faster isolate, SghaCWD$^{md\text{-}f}$, and with the slower isolates first passaged through the Tg (haPrP) mice, i.e., SghaCWD$^{e\text{-}s}$, SghaCWD$^{md\text{-}s}$, and SghaCWD$^{wd\text{-}s}$, revealed different patterns of PrP-res accumulation and gliosis (Fig. 5, PrP-res and GFAP panels, respectively). PrP-res was detected using 3F4 Ab, and gliosis was detected with anti-GFAP Ab as described in Materials and Methods. Immunohistological data are shown for sagittal sections of brains of an uninfected (normal) Sg hamster and a terminally affected 263K Sg hamster, SghaCWD$^{md\text{-}f}$, which was generated by serial passage of mdCWD through two Sg hamsters, and SghaCWD$^{md\text{-}s}$, which was generated by sequential passages, first into Tg (haPrP) mice and then into Sg hamster. Both PrP-res deposition and gliosis were widespread in the brains of 263K- and SghaCWD$^{md\text{-}f}$-affected hamsters, whereas in the SghaCWD$^{md\text{-}s}$-affected hamster they were not. The hippocampus, cerebellum, and caudal colliculus regions are also shown at higher magnification for more detailed analysis. SghaCWD$^{e\text{-}s}$ and SghaCWD$^{wd\text{-}s}$ showed patterns of PrP-res deposition and gliosis indistinguishable from those of SghaCWD$^{md\text{-}s}$ shown in Fig. 5 (data not shown).

Passages of CWD into various hamster species. In order to determine whether other hamster species may be susceptible to CWD and result in a useful rodent-adapted CWD animal model, brain homogenates isolated from elk, mule deer, or white-tailed deer brain pools were inoculated into several other hamster species (Table 3). In contrast to the transmission experiments using CWD isolates from individual elk, mule, and white-tailed deer (Table 1), none of the Sg hamsters inoculated with the CWD brain pools developed clinical disease within their life span, and no PrP-res was detected by immunoblot analysis of their brains. There was also no evidence of transmission to Djungarian hamsters. In contrast, there were obvious clinical signs in a majority of Chinese hamsters inoculated with the e- and mdCWD brain pools, though not with the wdCWD pool. Most of the clinically positive Chinese hamster brains tested were PrP-res positive by immunoblot analysis. With Siberian hamsters, there was one animal that received the mdCWD pool inoculum that was positive for both neurological signs of TSE disease and PrP-res. Another Siberian hamster was confirmed to be positive from the white-tailed deer pool inoculum. A single Armenian hamster was clinically suspect and immunoblot positive for the eCWD pool inoculum. All of the clinically infected animals initially presented with rapid tremors and ataxia that progressively worsened for 2 to 4 weeks until the animals became recumbent and were euthanized.

Passages into wild-type mice. The inocula used for the RML outbred mice and C57BL10 inbred mice were from the CWD-affected individual animal brains and the CWD brain pools,

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TABLE 2. Serial passage details of individual CWD-positive cervid animal brain homogenates inoculated into Tg (haPrP) mice

CWD inoculum	Primary passage					Second passage					Third passage			
	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time (dpi) of TSE-positive sample (mean ± SD)	Time (dpi) of blot-positive samples (mean ± SD)	Donor animal inoculum[c]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time of animals with TSE signs (mean dpi ± SD)	Donor animal inoculum[c]	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	Incubation time of animals with TSE signs (mean dpi ± SD)
e	Tg (haPrP) mo	3/9	8/9	632 ± 89	585 ± 142	e 1	Tg (haPrP) mo	7/7	3/3	282 ± 39	e 1a	Tg (haPrP) mo	4/4	203 ± 9
											e 1b	Tg (haPrP) mo	5/5	202 ± 7
						e 1	Sg ha	5/6	4/5[d]	509 ± 70	e 1c	Sg ha	5/5	543 ± 39
											e 1d	Sg ha	5/5	526 ± 90
						e 2	Tg (haPrP) mo	8/8	3/3	215 ± 9	e 2a	Tg (haPrP) mo	4/4	210 ± 10
											e 2b	Tg (haPrP) mo	4/4	185 ± 16
						e 3	Tg (haPrP) mo	7/7	3/3	212 ± 10	e 3a	Tg (haPrP) mo	6/6	247 ± 36
											e 3b	Tg (haPrP) mo	3/3	204 ± 9
md	Tg (haPrP) mo	3/8	5/8	726 ± 47	668 ± 105	md 1	Tg (haPrP) mo	8/8	3/3	202 ± 8	md 1a	Tg (haPrP) mo	4/4	213 ± 5
											md 1b	Tg (haPrP) mo	4/4	259 ± 33
						md 1	Sg ha	7/7	7/7	485 ± 44	md 1c	Sg ha	4/4	544 ± 110
											md 1d	Sg ha	2/2	540 ± 614
						md 2	Tg (haPrP) mo	8/8	4/4	209 ± 28	md 2a	Tg (haPrP) mo	3/3	228 ± 15
											md 2b	Tg (haPrP) mo	4/4	212 ± 14
wd	Tg (haPrP) mo	4/9	8/9	632 ± 73	621 ± 80	wd 1	Tg (haPrP) mo	7/7	4/4	272 ± 62	wd 1a	Tg (haPrP) mo	4/4	212 ± 32
											wd 1b	Tg (haPrP) mo	4/4	237 ± 13
						wd 1	Sg ha	5/5	5/5	462 ± 3	wd 1c	Sg ha	3/3	408 ± 42
											wd 1d	Sg ha	4/4	436 ± 15

[a] Abbreviations used are the same as those in Table 1.

[b] For each group, 12 animals were inoculated for the first passage, 8 animals were inoculated for the second passage, and 4 to 6 animals were inoculated for the third passage. Intercurrent deaths, as defined in Table 1, are not included in these data.

[c] The code designates the individual Tg (haPrP) mouse or Sg hamster that was used as a source of inoculum in the passage specified. For example, "e 1" designates an individual TSE-positive Tg (haPrP) mouse that was inoculated with eCWD and was used as the source of inoculum for a second passage; "e 1a" designates an individual Tg (haPrP) mouse from the second passage that had been inoculated from the e 1 Tg (haPrP) mouse.

[d] The blot-negative animal (second passage, e 1 inoculated into Sg hamster) was also negative for neurological signs and likely died due to another undetermined cause.

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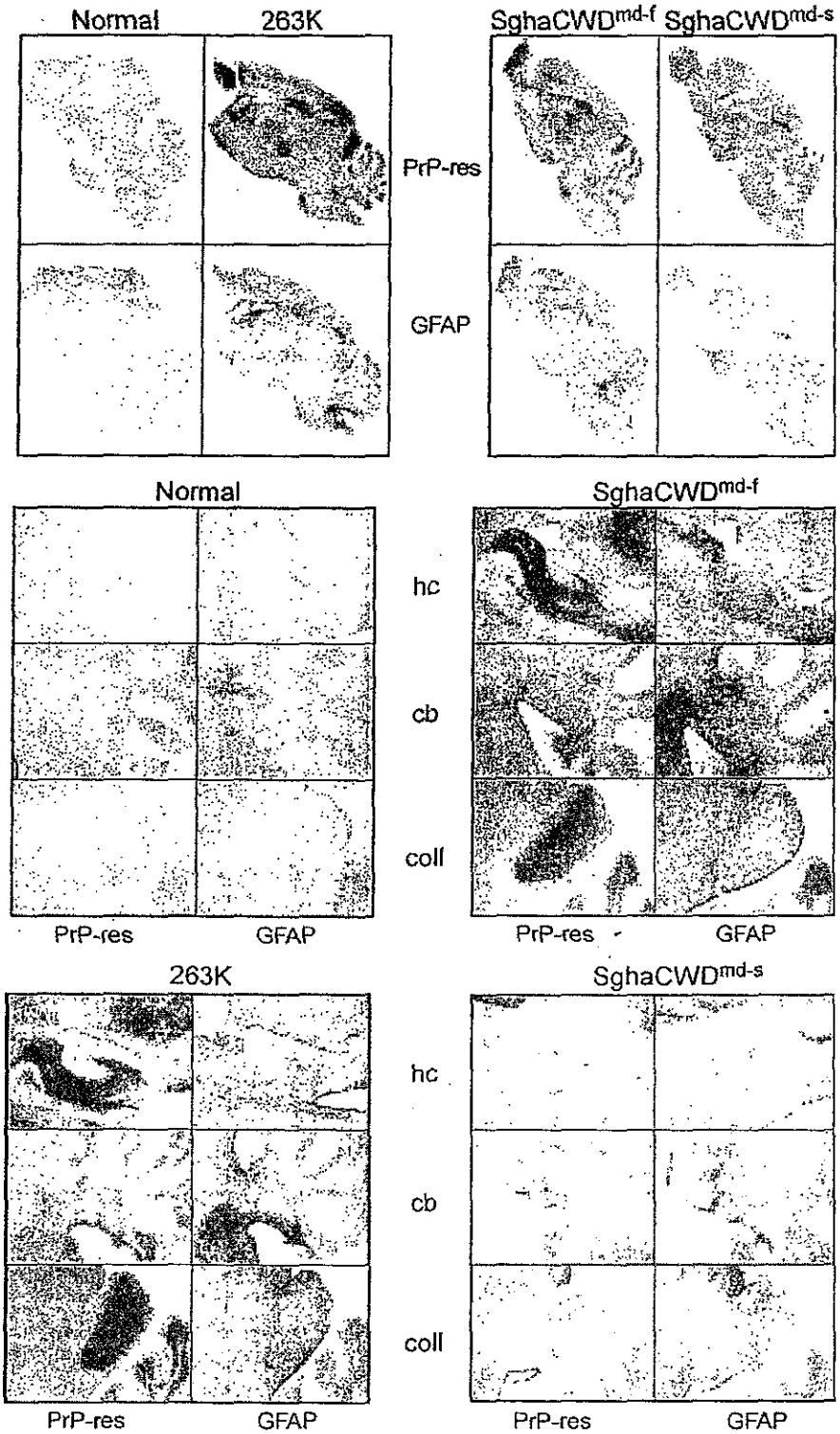

FIG. 5. Immunohistochemical analysis of brain sections from slow (SghaCWD^{md-s}) and fast (SghaCWD^{md-f}) isolates compared to uninfected (normal) and 263K-infected Sg hamster brains. PrP-res deposition was visualized in sagittal sections of the various brains, using 3F4 Ab ("PrP-res" panels). Using adjacent brain sections, the extent of gliosis was visualized using anti-GFAP Ab ("GFAP" panels). Scanned whole-brain images are shown in the upper panels. For the middle and lower panels, hippocampus (hc), cerebellum (cb), and caudal colliculus (coll) regions were magnified at ×40 for more detailed analysis. The images shown are representative of the following numbers of brains analyzed: two normal brains, two 263K-infected brains, three each of second- and third-passage SghaCWD^{md-f}-infected brains, and two each of third-passage SghaCWD^{md-s}-, SghaCWD^{o-s}-, and SghaCWD^{wd-s}-infected brains.

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TABLE 3. Passage details of CWD-positive cervid brain homogenates inoculated into various hamster and wild-type mouse species

CWD inoculum	Species inoculated[a]	No. of animals with TSE signs/no. inoculated[b]	No. of blot-positive animals/no. tested	Incubation time of animals with TSE signs (mean dpi ± SD)[c]	Life span (dpi, mean ± SD)	Life span range (dpi)
e	Sg ha	0/12	0/2	None positive	628 ± 98	480–751
md	Sg ha	0/12	0/2	None positive	591 ± 98	469–693
wd	Sg ha	0/12	0/3	None positive	510 ± 95	430–644
e	Djun ha	0/4	Not done	None positive	500 ± 95	384–615
md	Djun ha	0/10	Not done	None positive	507 ± 97	399–659
wd	Djun ha	0/1	Not done	None positive	476	476
e	Chin ha	6/8	4/5[e]	626 ± 80	730 ± 220	555–1,133
md	Chin ha	6/8	6/7[e]	648 ± 58	690 ± 166	563–1,133
wd	Chin ha	0/6	Not done	None positive	722 ± 239	448–1,133
e	Sib ha	0/2	Not done	None positive	532 ± 69	483–581
md	Sib ha	1/3	1/3[e]	464	482 ± 23	464–509
wd	Sib ha	1/4	1/4[e]	735	638 ± 118	499–739
e	Arm ha	1/4	1/2[e]	745	669 ± 121	490–745
md	Arm ha	0/15	0/1	None positive	625 ± 96	348–704
wd	Arm ha	0/8	0/8	None positive	587 ± 40	532–649
e[d]	RML mo	0/9	0/9	None positive	592 ± 80	488–736
md[d]	RML mo	0/6	0/6	None positive	679 ± 93	488–805
wd[d]	RML mo	0/6	0/6	None positive	730 ± 101	562–893
e	C57BL10 mo	0/15	0/2	None positive	724 ± 107	588–837
md	C57BL10 mo	0/16	0/2	None positive	657 ± 169	368–837
wd	C57BL10 mo	0/14	0/2	None positive	772 ± 125	368–837

[a] Abbreviations are the same as those in Table 1. Djun, Djungarian; Chin, Chinese; Sib, Siberian; Arm, Armenian; RML mo, Rocky Mountain Laboratory mouse.
[b] For each group, 12 to 16 animals were inoculated. Animals lost due to intercurrent deaths (as defined in Table 1) are not included in these data.
[c] None positive, none of the animals in the group were TSE positive by either neurological signs or immunoblot analysis for brain PrP-res during their life span.
[d] RML mice were inoculated with the individual CWD-positive cervid brain homogenates used for both the Sg hamsters in Table 1 and the Tg (haPrP) mice in Table 2.
[e] The blot-positive animals also showed neurological signs consistent with TSE disease, while the blot-negative animals did not.

respectively (Table 3). None of the animals of either mouse species developed any neurological signs within their life span (ranging from 488 to 893 dpi), and none were immunoblot positive for brain PrP-res.

DISCUSSION

We have shown that CWD from one or more cervid species can be transmitted to Sg, Chinese, Siberian, and Armenian hamsters and to Tg mice that express Sg hamster prion protein. Transmission of CWD to Sg hamsters was attempted previously without generating disease, most likely because the animals in that study were incubated for only 1 year (2). In the present study, inoculated animals were observed for periods exceeding 2 years for some animals. We found CWD transmission, with the highest attack rates in Chinese hamsters and Tg (haPrP) mice. The other rodent species had much lower attack rates or were not susceptible. The incomplete attack rates for the hamster species and Tg (haPrP) mice indicated that the cervid CWD inocula contained an average of only roughly 1 ID_{50} (the dose that would infect 50% of the animals) for these species. However, these inocula produced disease in Tg mice expressing deer PrP at a 100% attack rate, indicating that the titer for mice with homologous PrP is greater (R. E. Race, K. Meade-White, and B. Chesebro, unpublished data). The lack of transmission of some of the cervid CWD inocula to the other rodent species could be due to small differences in inoculum titers or to heterogeneity in the PrP sequences in the pooled inocula rather than to fundamental differences in host susceptibility. The amounts of normal host PrP expressed in the different hamster species are similar (K. Meade-White and R. E. Race, unpublished data) and are not likely an explanation for the different susceptibilities. Due to the low attack rates and long incubation periods seen with primary passages from cervids, none of these rodent species would be practical for use in direct bioassays for cervid CWD.

Nonetheless, the rodent-adapted CWD models we have developed may be useful to experimentally analyze TSE species and strain differences. Despite the low initial attack rates for the first passage of CWD into Sg hamsters, CWD isolates derived initially from elk and mule deer readily adapted to hamsters, as evidenced by the 100% infection rate on second and third passages. The average incubation periods were similar for the second and third passages but considerably shorter than that for the first passage for the Sg hamsters, suggesting that any species barrier to infection (formally, the shortening of the incubation period between the first and subsequent passages in a new species) was overcome quickly.

When mdCWD was serially passaged in Sg hamsters, an isolate, $SghaCWD^{md\text{-}f}$, was obtained that had a relatively short incubation period. When the same inoculum was passaged first into Tg (haPrP) mice followed by serial passage in Sg hamsters, an isolate with a fivefold longer incubation period developed,

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namely, SghaCWD$^{md\text{-}s}$. The CWD inocula from elk and white-tailed deer led only to the slow isolates SghaCWD$^{e\text{-}s}$ and SghaCWD$^{wd\text{-}s}$, which were indistinguishable from the slow mule deer isolate SghaCWD$^{md\text{-}s}$. The markedly different incubation periods of these two isolates from the mdCWD inoculum, as well as the distinct clinical signs and patterns of brain pathology and PrP-res deposition, raise the possibility that different strains of CWD isolates may exist, at least in mule deer, which in turn can lead to distinct CWD strains in Sg hamsters. Another possibility is that the strains diverged upon introduction into Sg hamsters, as suggested for the HY and DY Sg hamster strains from TME inoculum-infected mink brain homogenates (1).

Differences in PrP-res glycoform patterns analyzed from several CWD-affected deer and elk also suggested that CWD strains in mule deer may be more heterogeneous than those in elk (19). Others have also found evidence of CWD strains (16a). Curiously, however, this apparent strain difference was not manifested when the identical mdCWD inoculum was serially passaged through only one recipient species. Serial passage in Sg hamsters yielded only the fast-growing isolate (Table 1 and Fig. 3), while passage first through Tg (haPrP) mice and then into Sg hamsters yielded only the slow-growing isolate (Table 2 and Fig. 3). With this in mind, it is important to consider other possible explanations for these results. One possibility is that the CWD isolate might be able to undergo a stochastic change into a more rapid and aggressive strain in Sg hamsters and that this happened to occur after the mdCWD inoculations. This would be similar to the emergence of fast (HY) and slow (DY) strains upon inoculation of TME isolates into Sg hamsters (5). These strains developed even when a clonal isolate of the TME inoculum was used, suggesting that they arose in the recipient Sg hamsters rather than in the mink source (1).

Finally, although extensive precautions were taken, we cannot formally prove that inadvertent contamination of the mdCWD inoculum with the hamster-derived 263K strain did not occur, which potentially could yield short incubation period passages in Sg hamsters (Table 1). However, the incubation periods observed with the CWD passages (85 to 89 days) were significantly longer than the 263K incubation periods observed in our lab (70 to 75 days), and no mock-infected controls became sick during their life span. Also, we saw no 263K-like infectivity develop in the highly susceptible Tg (haPrP) mice, even though we used the identical primary inoculum for both recipient species. Interestingly, the similarity of the Sg hamster-adapted CWD fast-growing isolate and 263K might be due to a common origin, since there is circumstantial evidence that CWD arose from cervid exposure to sheep scrapie, which was also the origin of the 263K strain in hamsters (14). Furthermore, the Hyper strain derived from TME inoculations has 263K-like strain characteristics in Sg hamsters (5). Thus, it would appear that both CWD and TME transmissions into Sg hamsters can result in divergent fast and slow strains.

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医薬品
医薬部外品　研究報告　調査報告書
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別紙 3-3

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 6 月 14 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			研究報告の公表状況	Scrapie agent (strain 263K) can transmit disease via the oral route after persistence in soil over years. Seidel, B. et al., PLoS One, Issue 5, May 2007.	公表国 ドイツ	
販売名（企業名）						

研究報告の概要

ヒツジのスクレイピー自然伝播において，汚染された土壌が最も可能性の高い感染源の 1 つであると長い間疑われている。本試験では，環境における病原性のプリオン蛋白（PrP）の経時的な残留程度を測定し，シリアンハムスターにおける経口経路の伝播性をバイオアッセイにより検討した。これを実施するため，スクレイピー感染ハムスターの脳ホモジネートを混ぜた土壌検体をガーゼのバッグに入れ，15～20 cm の深さに埋めた。埋めた後に様々な時点において検体を掘り出し，ウェスタンブロット法により蛋白質分解酵素耐性 PrP^{Sc} の有無を調べた。その結果，異常な PrP は土壌中に残留し，29 ヵ月経過後にウェスタンブロット法によって検出されたことが示されたが，抽出量は経時的に低下した。21 ヵ月間土壌中に存在した折りたたみ異常プリオン蛋白は，蛋白折りたたみ異常反復増幅（PMCA）反応により蛋白分解酵素耐性 PrP の増幅を促進することができた。抽出された PrP はその生物学的活性を保持しており，131 日以内の感染土壌を摂餌した全てのシリアンハムスターおいてスクレイピーが誘発した。死亡時，これらの動物の脳には大量の異常な PrP が含まれていたことが示された。汚染された土壌の水溶性の抽出物も一部の動物でスクレイピーを引き起こしたが，この場合の潜伏期間は長かった。土壌から抽出された病原性 PrP に対して PMCA 反応を用いることができたことにより，環境におけるプリオン検出に新たな道が開かれると考えられる。

使用上の注意記載状況・その他参考事項等

BYL-2007-0288

報告企業の意見	今後の対応
スクレイピー物質は土壌中では長期にわたり比較的安定しており，ハムスターでの経口感染の可能性が明らかになっている。さらに，このメカニズムはシカの慢性消耗性疾患などその他のプリオン病の自然伝播にも関連している可能性がある。様々な感染源から，PMCA 反応は異常 PrP の検出に非常に有用であることが証明できるかもしれない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

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Scrapie Agent (Strain 263K) Can Transmit Disease via the Oral Route after Persistence in Soil over Years

Bjoern Seidel[1☯*], Achim Thomzig[2☯], Anne Buschmann[3☯], Martin H. Groschup[3], Rainer Peters[1], Michael Beekes[2], Konstantin Terytze[4]

1 Fraunhofer Institute for Molecular Biology und Applied Ecology (IME), Schmallenberg, Germany, 2 P24 -Transmissible Spongiform Encephalopathies, Robert Koch-Institut, Berlin, Germany, 3 Institute for Novel and Emerging Infectious Diseases, Friedrich-Loeffler-Institut, Insel Riems, Germany, 4 German Federal Environmental Agency (Umweltbundesamt, UBA), Dessau, Germany

The persistence of infectious biomolecules in soil constitutes a substantial challenge. This holds particularly true with respect to prions, the causative agents of transmissible spongiform encephalopathies (TSEs) such as scrapie, bovine spongiform encephalopathy (BSE), or chronic wasting disease (CWD). Various studies have indicated that prions are able to persist in soil for years without losing their pathogenic activity. Dissemination of prions into the environment can occur from several sources, e.g., infectious placenta or amniotic fluid of sheep. Furthermore, environmental contamination by saliva, excrements or non-sterilized agricultural organic fertilizer is conceivable. Natural transmission of scrapie in the field seems to occur via the alimentary tract in the majority of cases, and scrapie-free sheep flocks can become infected on pastures where outbreaks of scrapie had been observed before. These findings point to a sustained contagion in the environment, and notably the soil. By using outdoor lysimeters, we simulated a contamination of standard soil with hamster-adapted 263K scrapie prions, and analyzed the presence and biological activity of the soil-associated PrP^{Sc} and infectivity by Western blotting and hamster bioassay, respectively. Our results showed that 263K scrapie agent can persist in soil at least over 29 months. Strikingly, not only the contaminated soil itself retained high levels of infectivity, as evidenced by oral administration to Syrian hamsters, but also feeding of aqueous soil extracts was able to induce disease in the reporter animals. We could also demonstrate that PrP^{Sc} in soil, extracted after 21 months, provides a catalytically active seed in the protein misfolding cyclic amplification (PMCA) reaction. PMCA opens therefore a perspective for considerably improving the detectability of prions in soil samples from the field.

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INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) comprise a group of fatal neurodegenerative diseases such as bovine spongiform encephalopathy (BSE) in cows [1], chronic wasting disease (CWD) in deer (*Odocoileus* spp.) and elk (*Cervus elaphus nelsoni*) [2–4], scrapie in sheep and goats [5–7] and Creutzfeldt-Jakob disease (CJD) in humans [1]. The exact molecular pathomechanisms underlying TSEs have not yet been fully elucidated but it is generally accepted that a pathologically misfolded and/or aggregated isoform of the normal cellular prion protein (PrP), referred to as PrP^{Sc} and PrP^{C}, respectively, is the key pathogenic factor for this group of diseases [1].

Among the known TSEs, only scrapie and CWD are contagious diseases which show horizontal transmissibility under natural conditions [2,4,8]. CWD is the only TSE known to affect free-ranging animals [3]. The regular occurrence of scrapie in affected areas [7] and the spread of CWD in North America and Korea [9,10] among mule deer, white-tailed deer and elk indicates that a contagion in the environment is responsible for the occurrence of these TSEs [4,11–14], and even raises the possibility of a cross-species transmission under natural conditions. Recent findings demonstrated that saliva from deer with CWD harbours infectivity and can transmit this TSE upon peroral uptake [15]. Other studies pointed to transmission of scrapie among sheep by vectors like mites, fly larvae or other ectoparasites [16–19]. It has also been hypothesized that sporadically occurring TSEs may be induced by insecticides or by a disproportion of manganese and copper in soil leading to an enrichment of manganese in animals [20,21]. Alternatively, an influence of the Fe/Mn ratio in forage has been discussed in connection with TSEs [22]. However, on the balance of all evidence available so far, contaminated soil appears as one of the most likely sources of infection in the natural transmission of scrapie and possibly also CWD. It has been known for decades that sheep can become infected with scrapie while grazing on pastures where infected sheep have been kept before, and scrapie occurs often in areas where it has already occurred previously [7,8,23]. Furthermore, Brown and Gajdusek found that scrapie agent remains infectious after persisting in soil for 3 years as evidenced by intracerebral bioassay in Syrian hamsters [24]. The putative transmission of scrapie and CWD via soil is also corroborated by recent studies showing that prion infectivity binds to soil components with high affinity [25,26], thereby retaining its pathogenic biological activity [25]. Thus, soil-associated TSE agents in the environment represent a potential hazard. This holds true not least since prion infectivity exhibits an unusually pronounced resistance against both physical and

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* To whom correspondence should be addressed. E-mail: bjoern.seidel@ime.fraunhofer.de

☯ These authors contributed equally to this work.

chemical methods of inactivation, as described in detail elsewhere [27–37].

The contamination of soil with TSE infectivity can occur from several sources. Since recent studies could demonstrate that scrapie infectivity is present in various tissues and body fluids of infected animals [38,39], it has been assumed that the persistent prion protein enters the environment by contaminated excrements, birth-related tissues such as placenta, or even whole carcasses. While a similar excretion pattern appears conceivable for the CWD agent [40] which has also been found in saliva [15], the present knowledge about the BSE pathology in cattle does not argue for a significant shedding of the infectivity via faeces, urine, or during birth [41,42]. However, small ruminants infected with BSE could supposedly spread the BSE agent throughout the environment in a similar manner as known from scrapie-affected sheep or CWD-affected animals [43]. Moreover, unconventional conditions like for example the burial of animal carcasses at larger numbers as practiced for example during the foot and mouth disease outbreak in the UK in 2001 [44], may have fostered a dissemination of BSE agent in the soil or ground water. Once deposited there, all three TSE agents, BSE, scrapie, and CWD, must be assumed to persist in an infectious state for long periods of time.

So far, the oral transmission efficacy of long-term prion contaminations in soil have not been investigated. Therefore, we have studied the persistence of PrP^{Sc} in the environment over time and measured its oral transmissibility by bioassay in Syrian hamsters. With outdoor lysimeter experiments we simulated the situation on pastures using soil spiked with scrapie-infected hamster brain homogenate over a period of 29 months and analyzed the fate of the prion proteins by sensitive Western blotting and, in part, also by protein misfolding cyclic amplification (PMCA). The infectivity of such contaminated soil samples and the respective aqueous soil extracts was tested in the hamster bioassay.

RESULTS

Extraction and Recovery

In the first phase of the study several buffers – known as standard buffers for protein extraction from mammalian cells – and other solutions were tested in order to find out the optimal extraction method for prion protein from contaminated soil (see Materials and Methods). The obtained results lead us to use 1% SDS (sodium dodecyl sulphate) in sterilized water (figure 1a), which allowed detection of PrP^{Sc} in samples of German standard soil containing an amount of PrP^{Sc} that corresponded to 1.25 µg of infectious brain material (figure 1b).

Soil Incubation Experiments

The findings from our lysimeter experiments indicated a remarkable persistence of PrP^{Sc} in soil by clearly showing that - even after an incubation for 29 months - PrP^{Sc} could be still extracted from soil and detected by Western blotting. These results were confirmed by using two alternative highly sensitive Western blotting techniques. As shown in figure 2a, PrP^{Sc}, in the form of its protease-resistant core of the pathological prion protein PrP^{Sc} (PrP27-30), is still detectable in soil after 29 months. However, a decrease in the extractable concentration is obvious. The strongest decrease can be seen during the first month of incubation in the soil (figure 2a, lane 2 and 3). After three months the extractable amount of PrP27-30 remained almost constant until month 21, with small variations at month 12 and month 18 (figure 2a, lane 6 and 7). In Fig. 2a, a slightly stronger signal for

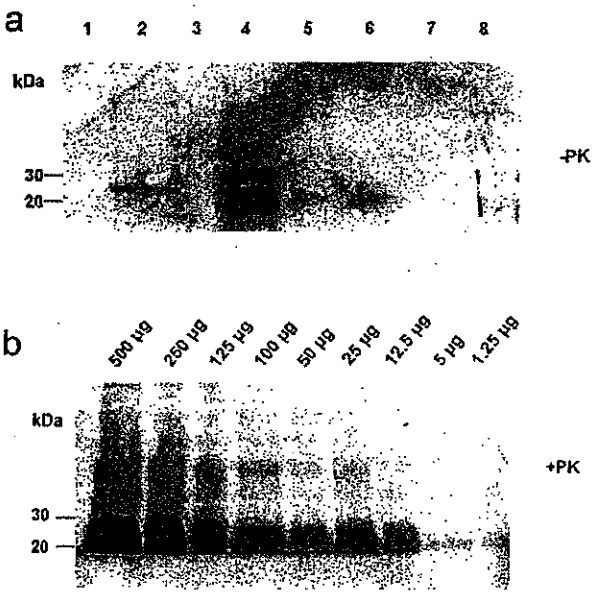

Figure 1. Western blot analysis of short-time incubation experiments. a) Western blot detection of PrP^{C} extracted from soil mixed with non-infectious brain homogenate (5% pork brain in German standard soil). Several different buffers and solutions were used for extraction. Lane 1: water; lane 2: Triton X-100; lane 3: 1% urea; lane 4: 1% SDS; lane 5: Zwittergent; lane 6: RIPA buffer; lane 7: NP-40; lane 8: Na-sarcosyl. b) Western blot detection of PrP27-30, the proteinase K-resistant core of PrP^{Sc}, extracted by using 1% SDS from soil contaminated with 263 K scrapie brain homogenate from hamsters after 1 h of incubation (dilution series). PrP^{Sc} could be detected in soil samples containing 1.25 µg or higher amounts of scrapie brain tissue after extraction with 1% SDS-solution. Samples were digested with proteinase K prior to Western blotting.
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PrP27-30 was found for 21 months (lane 8) as compared to 18 months (lane 7). Both samples were taken from the same soil bag, however, the location from where the sampling was performed may have differed. Thus, the finding possibly reflects minor inhomogenities either in the original load of contamination or in the degradation of PrP^{Sc} depending on the microenvironment of the examined sample. On the other hand, it has to be noted that the extraction yield and Western blotting efficiency inevitably may also vary in certain ranges from sample to sample. A further decrease was observed after 26 and 29 months (figure 1a, lane 9 and 10), however, the PrP27-30 specific bands remain clearly visible. Where performed, analyses of samples from different soil bags produced consistent results. In order to verify that the detected bands originated from PrP^{Sc} a control experiment was performed: After deglycosylation with PNGase F the PrP27-30 bands extracted from soil after 21 and 18 month showed an electrophoretic shift towards a single band at about 20 kDa, the molecular mass to be expected for deglycosylated PrP27-30 from 263K hamster scrapie (figures 2 b, lanes 3 and 4, respectively).

Amplification of PrP^{Sc} extracted from soil

In order to check whether PrP^{Sc} extracted from soil can be used for the amplification of protease resistant prion protein by the protein misfolding cyclic amplification (PMCA) reaction [45,46], soil extracts from month 21 were used as test seeds. As shown in figure 2c, a strong increase of the signal for PrP^{Sc} was obtained with this method: while no signal for PrP^{Sc} can be detected in

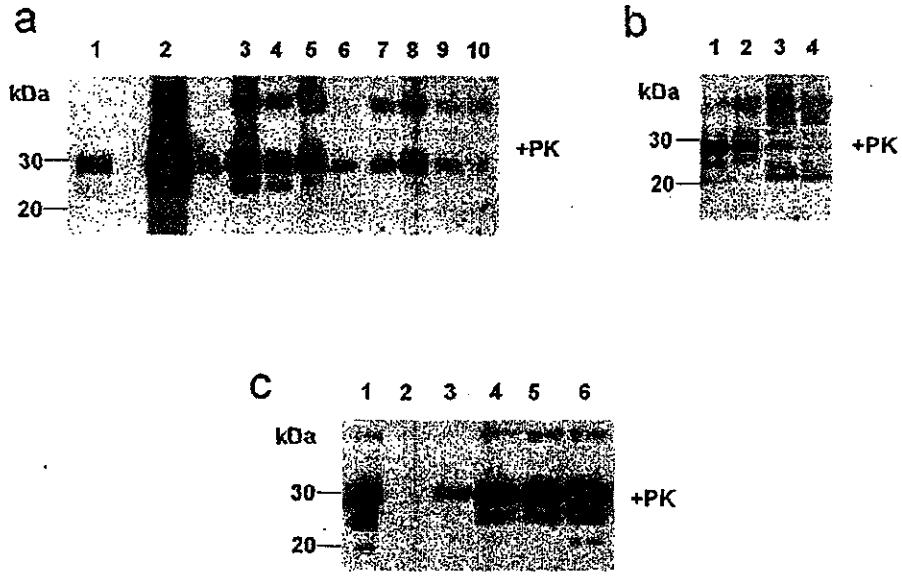

Figure 2. Western blot analysis of long-time incubation experiments. a) Western blot detection of PrP27-30 extracted from prion-contaminated soil after different time periods. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lanes 2–10: PrP27-30 extracted at time point 0 (lane 2), after 1 month (lane 3), after 3 months (lane 4), after 6 months (lane 5), after 12 months (lane 6), after 18 months (lane 7), after 21 months (lane 8), after 26 months (lane 9) and after 29 months (lane 10). **b)** Deglycosylated PrP27-30 extracted from prion contaminated soil. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lane 2: soil-extracted PrP27-30 after 21 months; lanes 3 and 4: deglycosylated soil-extracted PrP27-30 after 21 months (lane 3) and after 18 months (lane 4). **c)** PMCA amplification of PrP^{Sc} extracted from contaminated soil. Lane 1: PK-digested 263K scrapie hamster brain homogenate containing 5×10^{-7} g of brain tissue (positive control); lanes 2–6: sample signals after 0, 40, 80, 120 and 160 cycles of PMCA, respectively.
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samples without PMCA treatment (lane 2), clear signals became visible after 40 cycles of PMCA (lane 3) and even more intense after 80, 120 and 160 cycles of PMCA (lanes 4, 5 and 6, respectively).

Presence of PrP^{Sc} in surrounding soil samples

For analyzing the fate of the prion protein in more detail, soil samples in the vicinity of the gauze bag, as well as the gauze bag itself were analyzed by Western blotting. As shown in figure 3a, no PrP^{Sc} specific signal could be detected in the surrounding soil samples. This is indicative of PrP^{Sc} being immobilized after binding to soil compartments. In some approaches, a very faint PrP^{Sc} specific signal was visible when analyzing aliquots of SDS solution in which the gauze bag had been washed, however, it could be shown that this positive prion signal was a result of adherent soil particles (figure 3, lane 3, arrow).

Bioassay Experiments

For a detailed risk assessment of scrapie-contaminated soil it was of major importance to analyze whether the detectable PrP^{Sc} in the soil extracts still exhibited oral infectivity after incubation times up to 29 months. Therefore, a bioassay with Syrian hamsters was performed by feeding the animals with contaminated soil or aqueous soil extracts that had been collected after soil incubation for 26 and 29 months, respectively. Hamsters fed with contaminated soil exhibited first scrapie-associated symptoms at 131±6 days [mean±SD] after the first application. The hamsters reached the terminal stage of scrapie at 162±12 days after the first feeding (tables 1 & 2). This indicates substantial amounts of persistent infectivity in soil that had been incubated for 26 and 29 months. After reaching the terminal stage of scrapie the animals were sacrificed and analyzed for the presence of PrP^{Sc} in their brains. As shown in figure 4a (lanes 2 and 3 depicting two hamsters exemplarily), the brains of all animals in this group contained high amounts of PrP^{Sc}. In addition, comparison of the electrophoretic and glycosylation profiles of the brain samples from these hamsters (figure 4b, lanes 4 and 5) with those of hamsters challenged with different TSE isolates (figure 4b, ME7-H, lane 1; BSE-H, lane 2, 263K, lane 3) confirmed, that strain specific biochemical properties of the pathological prion protein

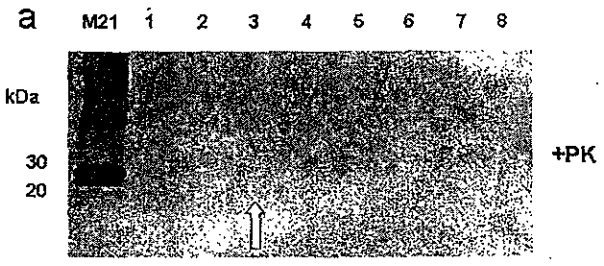

Figure 3. Western blot detection of PrP27-30 extracted from prion-contaminated soil after 21 months (M21) and respective surrounding samples. M21: contaminated soil sample inside the gauze bag; lane 1: soil sample collected outside of the steel cage; lane 2: soil sample collected directly over the gauze bag; lane 3: analysis of the empty gauze bag; lane 4: soil sample collected underneath the steel cage; lane 5: soil sample collected directly next to the gauze bag; lane 6: roots collected next to the gauze bag; lane 7: soil sample collected underneath the gauze bag; lane 8: non-contaminated soil. Arrow head at lane 3 indicates a faint PrP27-30 signal, resulting from residual soil particles that remained attached to the gauze bag.
doi:10.1371/journal.pone.0000435.g003

Table 1. Feeding schedule for bioassay in Syrian hamsters

Date	amount of soil or aqueous extract administered orally*	Collection time of soil sample
31/01/06	100 mg or 100 µl	month 26
07/02/06	100 mg or 100 µl	month 26
14/02/06	100 mg or 100 µl	month 26
21/02/06	100 mg or 100 µl	month 26
28/02/06	100 mg or 100 µl	month 26
08/03/06	100 mg or 100 µl	month 26
14/03/06	100 mg or 100 µl	month 26
21/03/06	50 mg or 50 µl	month 26
28/03/06	100 mg or 100 µl	month 29
06/04/06	100 mg or 100 µl	month 29
11/04/06	100 mg or 100 µl	month 29
20/04/06	100 mg or 100 µl	month 29

*mg relates to soil sample; µl relates to aqueous extract

doi:10.1371/journal.pone.0000435.t001

Table 2. Infection rate in hamster after oral challenge

Group	No. of animals infected with scrapie	Terminal stage of scrapie after the first oral application/Mean incubation period with the respective standard deviation in days
Control	0/5*	–
Control soil	0/5*	–
Control aqueous extract	0/5*	–
Contaminated soil	12/12	162 +/− 12
Contaminated aqueous soil extract	4/11*	256 +/− 41

*In the marked groups one animal each died at the beginning of the bioassay because of digestive disorders unrelated to scrapie disease

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were maintained in the animals fed with soil that had been incubated with 263K scrapie brain homogenate.

Remarkably, not only the hamsters fed with contaminated soil but also four hamsters of the group fed with aqueous soil extracts developed terminal scrapie at 256±41 days after the first application (with incubation times of individual animals ranging from 201 to 321 days). Since this bioassay is ongoing, further animals of this group might still develop scrapie in the future.

DISCUSSION

The results of this research project show for the first time that the scrapie strain 263K remains persistent in soil over a period of at least 29 months and remains highly infectious after oral application to Syrian hamsters. It has to be pointed out that the key results of our time-course study on the fate of PrP^{Sc} in soil have been validated, in part by examining blinded samples, at independent laboratories.

Only a few studies have addressed the question of a persistence of prions in soil so far [24–26,47], and the results from these studies are in principle in accordance with our observations. A pioneering study was published by Brown and Gajdusek in 1991 [24] showing that an aqueous extract from scrapie-contaminated soil remains infectious even after an incubation period of three years as confirmed by hamster bioassay. However, the infectivity studies were conducted by intracerebral injection and not by oral application. Furthermore, the PrP^{Sc} concentration was not analyzed in this study, so that no data are available about the proteins absorption behavior to soil particles and about the corresponding degradation kinetics. Most recently, PrP^{Sc} has been shown to bind to soil minerals [25] but only short-time incubation experiments of maximal one week were conducted and, again, bioassays were performed by the intracerebral route.

In this study we show by Western blotting a strong decrease in the amount of extractable PrP^{Sc} over an incubation period of 29 months in soil. It is not yet clear whether this decrease resulted from a molecular degradation of PrP^{Sc} or a tighter binding to soil particles. Stronger binding of molecules to soil particles with increasing incubation time is a well-known phenomenon in soil chemistry – the so called "aging" – and influences bioavailability and re-mobilization significantly [48,49].

Upon feeding hamsters with scrapie contaminated soil which had been incubated for over two years in outdoor lysimeters all animals developed terminal scrapie after relatively short in-

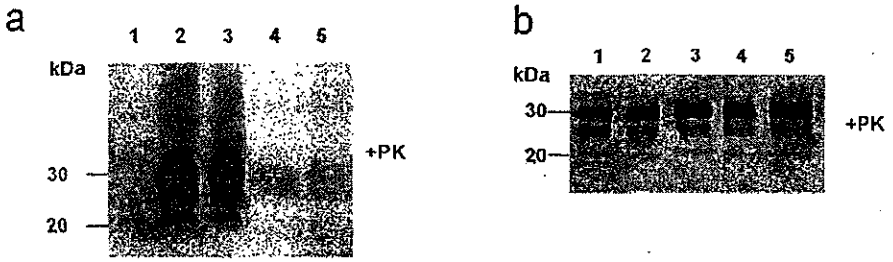

Figure 4. Western blot analysis of hamsters orally challenged with contaminated soil and western blot typing of PrP27-30. a) Western blot showing PrP^{Sc} in the brains of hamster orally challenged with contaminated soil samples and in the soil samples used for the bioassay. Lane 1: negative control hamster; lane 2: hamster H19 fed 12 times with aqueous extracts of soil samples from month 26 and 29; lane 3: hamster H4B fed 12 times with soil samples from month 26 and 29; lane 4: scrapie-contaminated soil (18 months); lane 5: scrapie-contaminated soil (21 months); lane 6: negative soil sample. b) Western blot typing of electrophoretic mobilities and glycosylation characteristics of PrP27-30 from different hamster-adapted TSE reference isolates, and from hamsters perorally challenged with 263K scrapie-contaminated soil. Lane 1: ME7-H scrapie agent; lane 2: hamster-adapted BSE-isolate (BSE-H); lane 3: 263K scrapie agent, lane 4: H10 (hamster 10, fed 12 times with soil samples from months 26 and 29); lane 5: H7 (hamster 7 fed 12 times with soil samples from month 26 and 29).

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cubation times (162 dpi). In other studies it has been well established that pure 10% (w/v) brain homogenates from 263K scrapie hamsters cause terminal scrapie in perorally challenged hamsters after mean incubation times of about 155–165 days with an attack rate of 100% [50–53]. This indicates that scrapie-contaminated soil may represent a potential TSE hazard for ruminants in the environment. While a considerable excretion of infectivity has to be assumed for scrapie or BSE infected sheep and CWD infected deer [40,43,54], it is generally acknowledged that the potential environmental contamination risk represented by BSE infected cattle is marginal, if at all present [41,42]. On the other hand, the burial of bovine carcasses [44] might have accidentally led to a spill of BSE prions into the environment. Furthermore, the fact that even feeding of aqueous extracts from scrapie-contaminated soil induced a terminal scrapie infection in four hamsters so far suggests that surface water or groundwater from pastures of scrapie-affected flocks may provide a potential source of scrapie infectivity.

However, the relevance of the results obtained in this study for the field situation should be interpreted with some caution, since only one soil type was used and only a limited number of animals were challenged in the bioassay. Therefore, other soil types and a larger number of animals have to be tested in future studies to allow for a robust risk assessment. Furthermore the exact binding properties and degradation kinetics of PrP^{Sc} should be subject to further research. In addition, all published studies addressing the persistence of prion infectivity in soil were performed with scrapie prions while TSE agents causing BSE and especially CWD have not been analyzed so far.

An intensified monitoring of PrP^{Sc} (and possibly also prion infectivity) in the soil appears mandatory for a more precise assessment of the risks emanating for humans and animals from prions in the environment. As shown in this report, PrP^{Sc} extracted from soil can be used as a catalytically active seed in the protein misfolding cyclic amplification (PMCA) reaction. This opens a promising perspective for considerably improving the detectability of prions in the environment.

MATERIALS AND METHODS

Biological Safety

Assays with scrapie-infected hamster brain were performed under laboratory conditions according to bio safety level 3** and in protected outdoor lysimeters, respectively.

Extraction of Brain Material

To identify a suitable buffer for extracting prion infectivity and PrP^{Sc} from soil, German standard soil (Lufa 2.2 and Borstel) was mixed with scrapie-infected hamster brain (strain 263K) provided by the TSE-Resource-Centre, Berkshire, Great Britain. To test the efficiency of this method the following solutions and buffers - especially non-ionic and ionic detergents - have been tested:

a) sterile water, b) 1% urea in sterile water c) 1% SDS in sterile water, d) 1% Zwittergent 3-08 in sterile water, e) 1% Triton X-100 sterile water, f) 10% Na-sarcosylate in sterile water, g) RIPA-buffer (0.25% Na-deoxycholate, 0.9% NaCl, 1% NOP-40, 0.8% Tris-HCl in sterile water (Carl Roth GmbH, Karlsruhe, Germany), h) 10% NOP-40 in sterile water, (Sigma-Aldrich, Steinheim, Germany).

Initially non-infectious pork brain was mixed with German standard soil and the efficiency of PrP^{C} extraction using the above mentioned buffers was tested. In a second step, the optimal extraction procedure was verified by applying the procedure to soil samples mixed with 263K scrapie agent and monitoring the PrP^{Sc} retrieval.

Incubation Experiments and Sampling Scheme

The outdoor experiments and all other experiments were performed using brains of terminally-diseased hamsters challenged with scrapie strain 263K.

For each approach, 1 g infectious hamster brain material was homogenized in 10 ml PBS (phosphate buffered saline) and added to 20 g of standard German sandy loom soil (Borstel). The soil/brain mixture was filled into gauze bags and buried in lysimeters filled with the same soil at a depth between 15 and 20 cm. The gauze had a mesh size of 250 µm, which enables the contact with microorganisms and meso fauna with the soil/brain mixture but avoids the contact with macro fauna derived/related organisms. To protect the gauze bags from mice, the bags were put into steel cages. At defined time points (after 0, 1, 3, 6, 12, 18, 21, 26, 29 months) the steel cages were dug out and the gauze bags as well as the surrounding soil were taken for sampling and analyzing. The analyses on the presence of residual PrP27-30 in soil samples were performed with a different number of soil bags, depending on the incubation times to be tested. For "short" incubation times of <12 months, for which detectability of residual PrP appeared most likely when the experiments were designed, samples from three different soil bags were examined. For "intermediate" incubation times of 12–18 months samples from two different bags were tested, and for "long" incubation times of >18 months one soil bag was yet available for sampling. All bags were buried in close vicinity.

Sample Preparation and Western Blot Analysis of Soil and Hamster Brain Samples

Remark: Western blot experiments were performed in independent runs at different laboratories, with each laboratory using its established techniques and procedures. The protocols are listed below. The different analytical protocols produced consistent results.

Western Protocol I

For the protein extraction from the contaminated soil as well as from surrounding soil samples, 20 ml of a 1%-SDS-solution (SDS; Sigma-Aldrich, Steinheim, Germany) were added to 20 g of testing material in a 50 ml tube. The suspension was vigorously shaken on a horizontal shaker for approx. 1 h, followed by a centrifugation step at 5,000 rpm for 20 min. 200 µl of the clear supernatant was incubated with proteinase K (50 µg/ml; 37°C; 1 h, Carl Roth GmbH, Karlsruhe, Germany) to eliminate non-resistant proteins. After digestion, the supernatants were boiled for 5 min in Laemmli's sampling buffer (50 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 50 mM ß-mercaptoethanol and 0.001% bromphenol blue) in a 1.5 ml tube and analyzed by Western blotting. The prepared samples were stored at −80°C.

For screening, Western blot samples were boiled for 5 min and separated by polyacrylamide gel electrophoresis (SDS-PAGE) by using 8–16% Tris-Glycine-SDS precast gels (i-Gels, Gradipore, LTF-Labortechnik, Wasserburg, Germany) or 4–20% precast gels (Precise Protein Gels, Perbio Science GmbH, Heidelberg, Germany) according to standard procedures as described previously [55–57]. After SDS-PAGE, the proteins were transferred to a polyvinylidene difluoride membrane (PVDF, Immobilon Millipore, Billerica, USA) using a semi-dry blotting system. Membranes were blocked in Superblock (Perbio Science GmbH, Heidelberg, Germany) with 0.05% Tween 20 over night at 4°C. Blots were incubated for 1 h in primary antibody solution (monoclonal anti-PrP 3F4, Chemicon International, Inc., California, dilution: 1:3,000 in PBS and 1.5% BSA or monoclonal anti-

PrP 3F4, Sigma-Aldrich, Steinheim, Germany, dilution: 1:4,000 in PBS and 1.5% BSA or monoclonal anti-mouse 6H4, Prionics AG, Zürich, Switzerland, 1:3,000 in PBS). After washing 5 times with PBS for 5 min and 0.05% Tween 20 (Perbio Science GmbH, Heidelberg, Germany), blots were incubated in a secondary antibody solution (horseradish peroxidase-conjugated anti-mouse, dilution 1:500,000 Perbio Science GmbH or alkaline-phosphatase-conjugated anti-mouse, dilution 1:5,000 Sigma-Aldrich, Steinheim, Germany) for at least 1.5 h. Membranes were washed as described before and developed with CDP-Star solution (Invitrogen GmbH, Karlsruhe, Germany) or SuperSignal West Femto Maximum Sensitivity substrate (Perbio Science GmbH, Heidelberg, Germany) for 5 to 10 min. Protein signals were visualized using Pierce film (Perbio Science GmbH, Heidelberg, Germany).

For confirmation of these results, highly sensitive Western blotting was performed in independent experiments at different laboratories using their established protocols.

Western Protocol II

The protein extractions from contaminated soil samples were carried out by adding 2 ml 1%-SDS-solution to 2 g of testing material in a 15 ml tube. The suspension was vigorously shaken for 2 min, followed by a centrifugation step at 7,000 rpm for 10 min. 50 µl of the clear supernatant was used for proteinase K digestion (100 µg/ml; 37°C; 1 h). After digestion the supernatants were mixed with an equal volume of 2× sample loading buffer (50 mM Tris (pH 6.8), 2% SDS, 10% glycerol, 50 mM ß-mercaptoethanol and 0.001% bromphenol blue) and heated to 100°C for 5 min for PrP-Western blotting.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyzes of samples from hamsters were performed as described elsewhere [58] with recently published modifications [39]. In short: Following SDS-PAGE, proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Immobilon, Millipore, Billerica, USA) using a semi-dry blotting system. The membrane was blocked by incubation for 30 min in TBS containing 3% (w/v) nonfat milk powder (NFMP) and 0.05% (w/v) Tween-20 (NFMP-TBST). Blots were incubated overnight in primary antibody solution (monoclonal anti-PrP antibody 3F4 [mAb 3F4] from cell culture, diluted 1:2,000 in NFMP-TBST) at 4°C. After washing 5 times for at least 20 min with NFMP-TBST, blots were incubated in secondary antibody solution (alkaline phosphatase conjugated goat anti-mouse IgG; Dako, Glostrup, Denmark; 1:5,000 diluted in NFMP-TBST) for 90 min at room temperature. After washing 5 times with NFMP-TBST over a total period of at least 1.5 h, the membranes were pre-incubated 2×5 min in assay-buffer (100 mM Tris, 100 mM NaCl, pH 9.5) and finally developed with CDP-star solution (Tropics, Applied Biosystems, Beford, USA) for 5 min according to the instructions of the manufacturer. PrP-signals were visualized on a X-OMAT AR (Kodak, Sigma-Aldrich, Steinheim, Germany) film. Films were exposed for 5–30 min.

For deglycosylation, 500 µl of the soil extract solution were dialyzed in PBS for 16 h in order to remove the detergents, and 20 µl of the aliquot was digested using PNGase F (New England Biolabs, Ipswich, USA) according to the instructions of the manufacturer prior to Western blotting.

Western Protocol III

Sample preparation and extraction of the prion protein from soil samples as well as proteinase K digestion was performed as described in protocol I. After electrophoresis using 16% bis-acrylamide gels, proteins were transferred on a PVDF-membrane in a semi-dry chamber. The membranes were blocked in 5% dry fat milk in PBS (phosphate buffered saline) containing 0.1% Tween 20 (Merck, Darmstadt, Germany) (PBS-T) for 30 min and subsequently incubated with the PrP-specific monoclonal detection antibody 3F4 (Chemicon International, Inc., California) in a dilution of 1:3,000 in 5% dry fat milk in PBS-T for 1 h 30 min. The membranes were washed 3 times for 10 min with PBS-T and then incubated with a secondary antibody bound to alkaline phosphatase in a dilution of 1:2,000 in PBS-T (goat-anti-mouse-AP, Dianova, Hamburg, Germany) for 1 h. After again washing three times for 10 min, the membranes were incubated 2 times for 2 min in assay buffer containing 200 mM Tris-HCl and 10 mM $MgCl_2$ (pH 9.8). Finally, the chemiluminescence substrate CDP-Star (Tropix, Bedford, USA) was applied and incubated on the membrane for 5 min before the light signals were detected in a camera using the analysis software Quantity One (Bio-Rad, Munich, Germany).

Bioassay in Syrian hamsters

Forty two female Syrian hamsters, approx. 90 days old, were obtained from Charles River Laboratories, Germany. They were handled according to the regulations of the local authorities (Bezirksregierung Arnsberg, reference number 50.8735.1 Nr. 108/1) in a biosafety Level 3 containment facility.

12 animals were fed weekly over a period of 12 weeks; 11 times with 100 mg soil/brain mixture each and one time with 50 mg soil/brain mixture taken from the outdoor lysimeters after an incubation period of 26 and 29 months (table 1). Oral application was achieved by mixing the soil (1,150 mg in total) with commercial hamster feed (ssniff, Soest, Germany).

To analyze the remaining infectivity in the aqueous extract, 10 g contaminated soil was mixed with 10 ml water and was vigorously shaken on a horizontal shaker for approx. 1 h. The mixture was centrifuged with 5,000 rpm for 5 min and the supernatant was used for the bioassay. Oral application was performed weekly over a period of 12 weeks (1,150 µl in total) by mixing the extract with commercial hamster feed (ssniff, Soest, Germany). For this purpose, 12 additional animals were fed 11 times with 100 µl and 1 time with 50 µl aqueous extract from the soil/brain mixture taken from the outdoor lysimeters after an incubation period of 26 and 29 months.

The hamsters of both groups were monitored at least twice a week for the development of clinical signs of scrapie. Hamsters diseased with 263K scrapie showed head bobbing, ataxia of gait and generalized tremor. Such animals were frequently and persistently in motion, easily irritated by noise and touch, upon which they often twitch, and had difficulties maintaining balance and rising from a supine position. These clinical symptoms of hamsters are entirely consistent with those previously reported for the 263K scrapie agent [59]. At the occurrence of first neurological symptoms, the animals were observed daily and at appropriate end-points, clinically affected hamsters were killed. Brains from these hamsters were examined and analyzed for the presence of PrP^{Sc}.

As a control, six animals were fed with non-contaminated standard soil and 6 animals were fed with aqueous extract from non-contaminated standard soil over a period of 12 weeks (1,150 mg or 1,150 µl in total). Additional 6 hamsters were fed only with commercial hamster feed (ssniff, Soest, Germany).

PMCA-Reaction

The PMCA method was carried out as reported previously [45,46] with modifications: In brief, normal hamster brains were

homogenized at 10% (w/v) in PBS containing complete protease inhibitor cocktail (Boehringer-Ingelheim, Mannheim, Germany), 4 mM EDTA and 1% Triton-X-100. The homogenate was briefly centrifuged at 2,000 g and the supernatant was used as the PrP^C source. Amplification was performed using an automatic ultrasonification apparatus (Sonicator 300 from Misonix, New York, USA) with a water-tank.

The PrP^C substrate was mixed with 1/10 volume of the soil extract previously dialyzed in PBS, resulting in a total volume of 100 µl. PMCA amplification was performed by 40 cycles of sonication (40 sec. each) followed by incubation at 37°C for 1 h in the water-tank. The amplified product of the first round of amplification was diluted 1:5 with normal brain homogenate and the second round was performed. This process was repeated 4 times to obtain 160 cycles of PMCA. From each PMCA-amplification round, aliquots of 50 µl were taken and digested with proteinase K (180 µg/ml) for 1 h at 56°C. Finally, the same volume of 2× sample buffer was added and heated for 10 min at 100°C prior to SDS-PAGE.

Western Blot Typing of PrP27-30

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyzes of samples from hamsters for characterization of the PrP^{Sc} glycosylation and migration pattern were performed as described elsewhere [59]. In short: 50 µl of 10% (w/v) brain homogenates in TBS (pH 7.4) were mixed with 5 µl of 13% (w/v) sarcosyl and 10 µl proteinase K stock solution (1 mg/ml; Roche, Mannheim, Germany) and digested for 60 min at 37°C. The digestion was stopped by adding 435 µl 2× sample buffer, i.e. 4% (w/v) SDS, 10% (v/v) 2-mercaptoethanol in 120 mM Tris-HCl, pH 6.8, containing 20% (w/v) glycerol and 0.05% (w/v) bromophenol blue, and boiling for 5 min. 5 µl of the solution (corresponding to 5×10^{-5} grams of homogenized brain tissue) were separated in a 15% SDS-PAGE or in Tris-glycine gels (Novex, Invitrogen, Carlsbad, USA) and subsequently blotted onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, USA) using the semi-dry method (Fast-Blot; Biometra, Göttingen, Germany). The PVDF-membranes were blocked with 5% low-fat milk in TBS for 30 min and incubated overnight at 4°C with MAb 3F4 (1:2,000) in 3% bovine serum albumin (BSA) in TBS. After washing in TBS and incubation for 60 min at room temperature with the secondary antibody [biotinylated goat anti-mouse IgG (1:2,000) in 3% BSA in TBS], a biotin-streptavidin kit (Dako, Glostrup, Denmark) for signal enhancement was applied for 30 min at room temperature. After washing the membranes in TBS, antibody binding to PrP was visualized using a mixture of nitroblue-tetrazoleum (NBT; Sigma-Aldrich, Steinheim, Germany) and 5-bromo-4-chloro-3-indolylphosphate (BCIP; Sigma-Aldrich, Steinheim, Germany) as substrate.

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Author Contributions

Conceived and designed the experiments: MB AT MG AB BS RP KT. Performed the experiments: AT AB BS RP. Analyzed the data: MB AT MG AB BS RP KT. Contributed reagents/materials/analysis tools: MB AT. Wrote the paper: MB AT MG AB BS KT.

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研究報告の概要

プリオン感染性及びその分子マーカーである病原性プリオン蛋白 PrP^{Sc} は、中枢神経系に蓄積するとともに、しばしばTSEに罹った動物又はヒトのリンパ組織にも蓄積する。最近、以前にはプリオンに侵入されないと考えられていた組織（例えば骨格筋）内に PrP^{Sc} が発見された。我々は、経口的又は非経口的にスクレイピーを投与したハムスターにおいて、プリオンが皮膚をターゲットにするか、臓器に PrP^{Sc} の蓄積が広い範囲にわたってみられるかという問題に取り組んだ。スクレイピーを経口的に投与したハムスターでは、PrP^{Sc} は症状発現前に検出されたが、皮膚に関係した PrP^{Sc} の大部分は、臨床症状が現れた段階で蓄積した。PrP^{Sc} は皮膚内部の神経線維に局在していたが、ケラチン生成細胞には局在しておらず、皮膚での PrP^{Sc} の蓄積は、感染の経路及びリンパ球向性の伝播に依存しないことを示した。このデータは、プリオンが神経によって仲介され、皮膚へ遠心的に拡散することを示した。さらに追跡試験によって我々は、スクレイピーに自然感染した羊を調べ、5頭の内2頭の皮膚の検体中からウエスタンブロットによって PrP^{Sc} が検出された。皮膚がプリオンの貯蔵臓器とする我々の所見について疾患の伝播と関連した調査をさらに実施する必要がある。

報告企業の意見	今後の対応
実験的感染及び自然感染によってスクレイピーに罹った動物の皮膚に病原性プリオン蛋白 PrP^{Sc} が蓄積したとの報告である。 これまで血漿分画製剤によってvCJD、スクレイピー及びCWDを含むプリオン病が伝播したとの報告はない。しかしながら、万一vCJD感染者の血漿が本剤の原料に混入した場合には、製造工程においてプリオンを低減し得るとの報告があるものの、製剤から伝播する可能性を完全には否定し得ない。そのため、弊社の血漿分画製剤の製造工程におけるTSE感染性低減に関する検証実験を加速し、自社データを早期に取得し、工程評価を行い、必要に応じて工程改善を実施する予定である。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
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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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Accumulation of Pathological Prion Protein PrP^{Sc} in the Skin of Animals with Experimental and Natural Scrapie

Achim Thomzig[1☯*], Walter Schulz-Schaeffer[2☯], Arne Wrede[2], Wilhelm Wemheuer[3], Bertram Brenig[3], Christine Kratzel[1], Karin Lemmer[1], Michael Beekes[1*]

1 P24 Transmissible Spongiform Encephalopathies, Robert Koch-Institut, Berlin, Germany, 2 Prion and Dementia Research Unit, Department of Neuropathology, Universitätsklinikum Göttingen, Göttingen, Germany, 3 Institute of Veterinary Medicine, Georg-August-Universität Göttingen, Göttingen, Germany

Prion infectivity and its molecular marker, the pathological prion protein PrP^{Sc}, accumulate in the central nervous system and often also in lymphoid tissue of animals or humans affected by transmissible spongiform encephalopathies. Recently, PrP^{Sc} was found in tissues previously considered not to be invaded by prions (e.g., skeletal muscles). Here, we address the question of whether prions target the skin and show widespread PrP^{Sc} deposition in this organ in hamsters perorally or parenterally challenged with scrapie. In hamsters fed with scrapie, PrP^{Sc} was detected before the onset of symptoms, but the bulk of skin-associated PrP^{Sc} accumulated in the clinical phase. PrP^{Sc} was localized in nerve fibres within the skin but not in keratinocytes, and the deposition of PrP^{Sc} in skin showed no dependence from the route of infection and lymphotropic dissemination. The data indicated a neurally mediated centrifugal spread of prions to the skin. Furthermore, in a follow-up study, we examined sheep naturally infected with scrapie and detected PrP^{Sc} by Western blotting in skin samples from two out of five animals. Our findings point to the skin as a potential reservoir of prions, which should be further investigated in relation to disease transmission.

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Introduction

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are fatal neurodegenerative diseases affecting both animals and humans. According to the prion hypothesis, TSEs are caused by infectious prions that consist essentially—if not entirely— of a misfolded form of the prion protein (PrP), which is known as PrP^{Sc} [1]. Although the precise molecular composition and structure of prions remains elusive, PrP^{Sc} has been shown in many studies to accumulate together with infectivity in target tissues of infection and is therefore considered a reliable biochemical marker for TSE agents [2] as reported for experimentally challenged hamsters [3], other animal species [4], and humans [5,6].

Scrapie of sheep and goats, chronic wasting disease (CWD) of deer, bovine spongiform encephalopathy (BSE) of cattle, and variant Creutzfeldt-Jakob disease (vCJD) of humans represent acquired prion diseases that are caused by exposure to TSE agents in the living environment of the respective host. Different lines of evidence suggest that many, if not the majority, of cases of ovine scrapie, BSE, and purportedly CWD are caused by ingestion of prions and subsequent invasion of the organism via the alimentary tract [7]. This also holds true for vCJD, which is now generally acknowledged to be acquired through consumption of BSE-contaminated foodstuffs [8].

Although the exact mechanism of infection following passage of prions through the alimentary tract has not yet been completely elucidated, findings from different mammalian species suggested that the infection ascended retrogradually via peripheral nerves to the spinal cord and to the brain (for reviews see [2,7]). From these sites of initial central nervous system invasion at the level of the thoracic spinal cord and the medulla oblongata, the infection propagated in both ascending and descending directions [2,3,7,9–11]. Centrifugal spread from the central nervous system appeared to be responsible for subsequent infection of further parts of the peripheral nervous system [9,11]. In particular, PrP^{Sc} was found associated with nerve fibres or nerve endings innervating peripheral organs and tissues such as muscles [11–14]. This prompted us to look for further tissues which could serve as reservoirs of prions in the mammalian body, and from which these pathogens could be potentially disseminated into the environment and transmitted to other individuals via peroral or alternative routes. In this context, the skin appears to be of utmost importance. The skin

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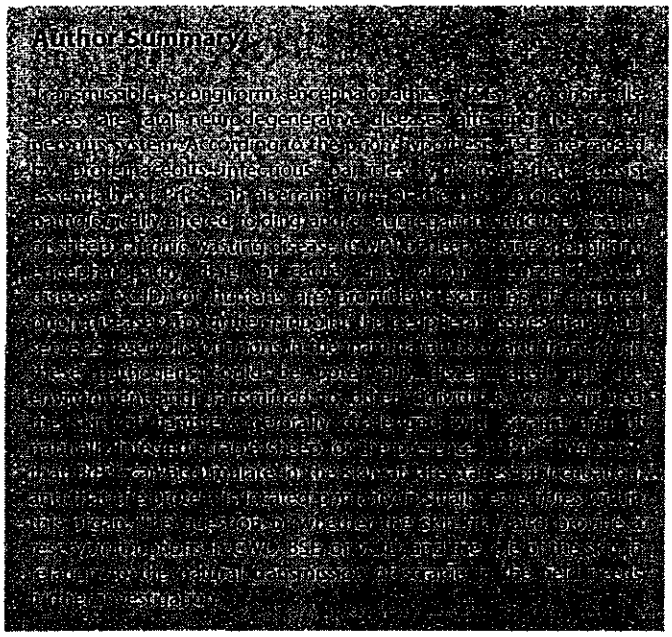
Abbreviations: BSE, bovine spongiform encephalopathy; BSE-H, hamster-adapted BSE; CWD, chronic wasting disease; dpi, days post infection; f.p., foot pad; H&E, haematoxylin and eosin; i.c., intracerebral; PET, paraffin-embedded tissue; p.o., oral; PrP, prion protein; PrP27–30, protease-resistant core of the pathological prion protein PrP^{Sc}; PrP^{C}, cellular isoform of the prion protein; PrP^{Sc}, pathological isoform of the prion protein; s.w., steel wires; TSE, transmissible spongiform encephalopathy; vCJD, variant Creutzfeldt-Jakob disease

* To whom correspondence should be addressed. E-mail: ThomzigA@rki.de (AT); BeekesM@rki.de (MB)

☯ These authors contributed equally to this work.



consists of different strata and appendages which are highly innervated and interspersed with lymphatics and blood vessels [15]. It constitutes the largest organ of humans and many animal species and provides an interface with their environment. However, although PrP^{Sc} detection has been reported for mucosal tissue [16,17], the skin has not been extensively studied for the presence of prions and PrP^{Sc} so far. In 2004, Cunningham et al. reported on the presence of BSE agent in a wide range of tissues from a BSE-infected greater kudu [18]. In one animal of this study, the salivary gland and skin were found to contain infectivity, and the authors suggested that these findings possibly indicate routes by which direct animal-to-animal transmission of the disease may occur.

Here, we examined the skin of prion-infected hamsters for the presence of PrP^{Sc}. Our hamster experiments focussed on orally infected animals, which have been previously established as a relevant rodent model to study the spread of prions in the peripheral nervous system [2,7]. These studies were performed in order to (i) investigate whether anatomical structures within the skin may provide a target for PrP^{Sc} accumulation, (ii) elucidate the identity of such skin components, and (iii) find out whether prions can be present in the skin prior to the onset of visible TSE symptoms. In a proof-of-concept approach, we extended PrP^{Sc} testing of the skin to specimens from sheep naturally infected with scrapie. This follow-up study intended to obtain further insights into the pathophysiology of scrapie and the putative pathways of its natural transmission in the field.

Results

PrP^{Sc} Accumulation in the Skin following Peroral Infection with Scrapie Becomes Detectable Shortly before the Onset of Clinical Symptoms

To investigate whether and at which stages of scrapie infection PrP^{Sc} accumulates in the skin, we performed a time-course study in hamsters orally exposed to 263K scrapie agent. As established previously for the examination of muscle tissue [11], PrP^{Sc} was visualized by sensitive Western blotting after extraction of the protein in the form of its protease-resistant core, PrP27–30, from skin specimens by a high-yield purification method.

We examined samples of skin tissue from hamsters taken at five different time points, i.e., at 70, 100, and 130 days post infection (dpi) in the pre-clinical phase of incubation, at the onset of clinical symptoms, and at the end stage of disease, which occurred after 164 ± 6 d (expressed as the mean ± standard deviation [SD]; $n = 5$). Skin samples from the *following five body regions of each hamster were analyzed:* the forelimb, the hindlimb, the abdomen, the back, and the head.

PrP27–30 could not be detected in any of the examined skin samples at 70 and 100 dpi from five animals each (Figure 1A and 1B). The earliest unambiguous signals for accumulation of the pathological prion protein PrP^{Sc} were found in skin samples from three out of five animals at 130 dpi in the late pre-clinical phase of incubation, corresponding to about 80% of the mean incubation period until terminal disease (Figure 1C, Western blot on the right-hand side, lanes S1, S2, S4, and S5). However, variable combinations of PrP^{Sc}-positive skin samples from different regions of the body were found at 130 dpi, indicating individual variation in the spread of infection to, or inhomogeneous distribution of PrP^{Sc} in skin tissue. Possibly, prion infection of the skin could have been detected more frequently in animals at 130 dpi, or at earlier pre-clinical stages of incubation, by using alternative methodologies such as the conformation-dependent immunoassay [19] or bioassays. At the onset of clinical symptoms, all of the analyzed skin specimens from all five examined hamsters displayed more or less strong signals for PrP27–30 (Figure 1D). At the terminal stage of scrapie, the positive signals for PrP27–30 become more intense, suggesting that accumulation of PrP^{Sc} takes place predominantly in relatively late stages of incubation (Figure 1E, lanes S1–S5). The weight of the tested skin samples ranged from approximately 40 to 100 mg as specified in the legend to Figure 1A–1E. In order to verify that the detected bands originated from PrP^{Sc}, a control experiment was performed: After deglycosylation with PNGaseF, the PrP27–30 bands showed an electrophoretic shift towards a single band at about 20 kDa, the molecular weight to be expected for the unglycosylated PrP27–30 form of 263K hamster scrapie (Figure 1F, lanes S1d–S5d). Control samples from mock-challenged age-matched hamsters consistently produced negative results (not shown). A time-scale displaying an overview of the time-points at which the p.o.-infected hamsters were tested for skin-associated PrP^{Sc} deposition in relation to the mean incubation period and the pre-clinical and clincal phases of incubation is provided in Figure 1G.

Location of PrP^{Sc} within the Skin

To determine where in the skin PrP^{Sc} accumulates, we investigated samples from the head, snout, forelimb, and abdomen of orally 263K scrapie–infected, terminally ill hamsters. As done previously when determining the route of PrP^{Sc} propagation to muscles [11], we used the paraffin-embedded tissue (PET) blot method to achieve a sensitive topographical localisation of disease-associated PrP in the skin. Using either Carnoy- or paraformaldehyde-fixed tissue

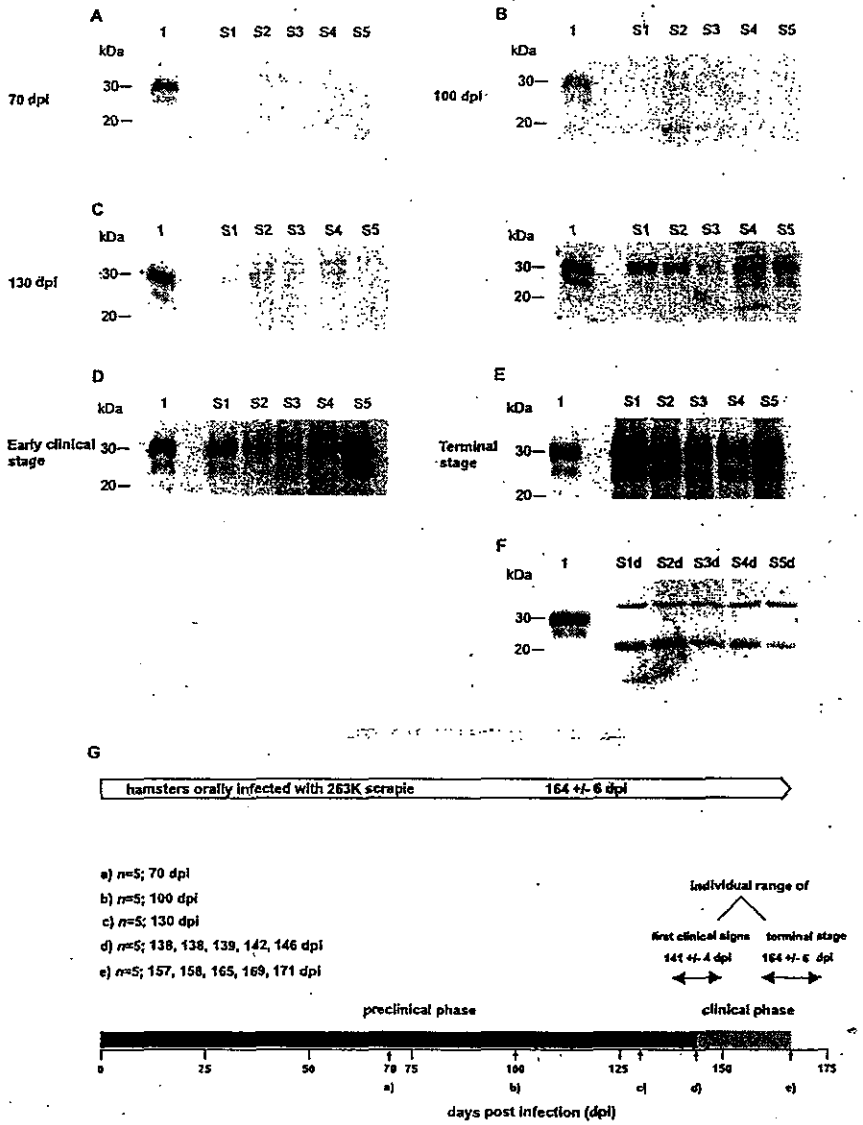


Figure 1. Time-Course of PrP^{Sc} Deposition in Skin Tissue

(A–E) Western blot detection of PrP27–30, the protease-resistant core of PrP^{Sc}, extracted from different skin samples of hamsters orally challenged with 263K scrapie and sacrificed at the following time-points after infection: (A) 70 dpi, (B) 100 dpi, (C) 130 dpi, (D) at the onset of clinical signs for scrapie (138–146 dpi), and (E) at the terminal stage of disease (157–171 dpi). Lanes with test samples: S1, skin sample from hindlimb; S2, skin sample from forelimb; S3, skin sample from back; S4, skin sample from abdomen; S5, skin sample from head. Lanes with control samples: 1, proteinase K–digested brain homogenate from terminally ill 263K scrapie hamsters containing 1×10^{-7} g brain tissue. Representative results are shown for each stage of incubation. Substantial individual variation was observed at 130 dpi, with two of five and three of five animals displaying findings as in (C) in the Western blot on the left-hand side or the Western blot on the right-hand side, respectively.

(F) Lanes S1d–S5d: Same samples as in S1–S5 of (E) but deglycosylated with PNGaseF.

(A–F) Amounts of tissue represented in lanes: (A) S1, 43 mg; S2, 52 mg; S3, 68 mg; S4, 58 mg; S5, 73 mg; (B) S1, 78 mg; S2, 44 mg; S3, 63 mg; S4, 67 mg; S5, 50 mg; ([C], Western blot on the left side) S1, 42 mg; S2, 76 mg; S3, 61 mg; S4, 58 mg; S5, 73 mg; ([C], Western blot on the right side) S1, 51 mg; S2, 63 mg; S3, 70 mg; S4, 87 mg; S5, 54 mg; (D) S1, 63 mg; S2, 68 mg; S3, 90 mg; S4, 50 mg; S5, 68 mg; (E) S1, 55 mg; S2, 73 mg; S3, 80 mg; S4, 88 mg; S5, 70 mg; (F) S1d, 12 mg; S2d, 14 mg; S3d, 19 mg; S4d, 12 mg; S5d, 20 mg.

(G) Time-scale displaying the mean incubation period and the pre-clinical and clinical phases of incubation of hamsters orally infected with 263K scrapie. Small vertical arrows indicate time-points at which animals were tested for PrP^{Sc} in skin samples.

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samples, PrP^{Sc} was detectable in (i) free nerve endings of the subepidermal plexus on the border of the epidermis to the dermis (Figure 2A, 2B, 2G, and 2H, arrows), (ii) fibres of the subepidermal, the deep cutaneous, and the subcutaneous plexus, (iii) fibres of the follicular neural network of the hair (circular and longitudinal fibres, Figure 2A, 2B, and 2G, arrowheads), (iv) the hair follicle isthmus (Figure 2G, rhombus), and (v) small intradermal striated fibres of mimic muscles (Figure 2A and 2B, asterisks). No PrP^{Sc} was detectable in keratinocytes, epidermal basal cells, fibroblasts of the dermal connective tissue, capillary blood vessels, outer root sheet cells of the hair, or the bulge region and the sebaceous gland, but PrP^{Sc} was present in nerve fibres of the sebaceous gland (not shown). Nerve fibres in the skin can be labelled by

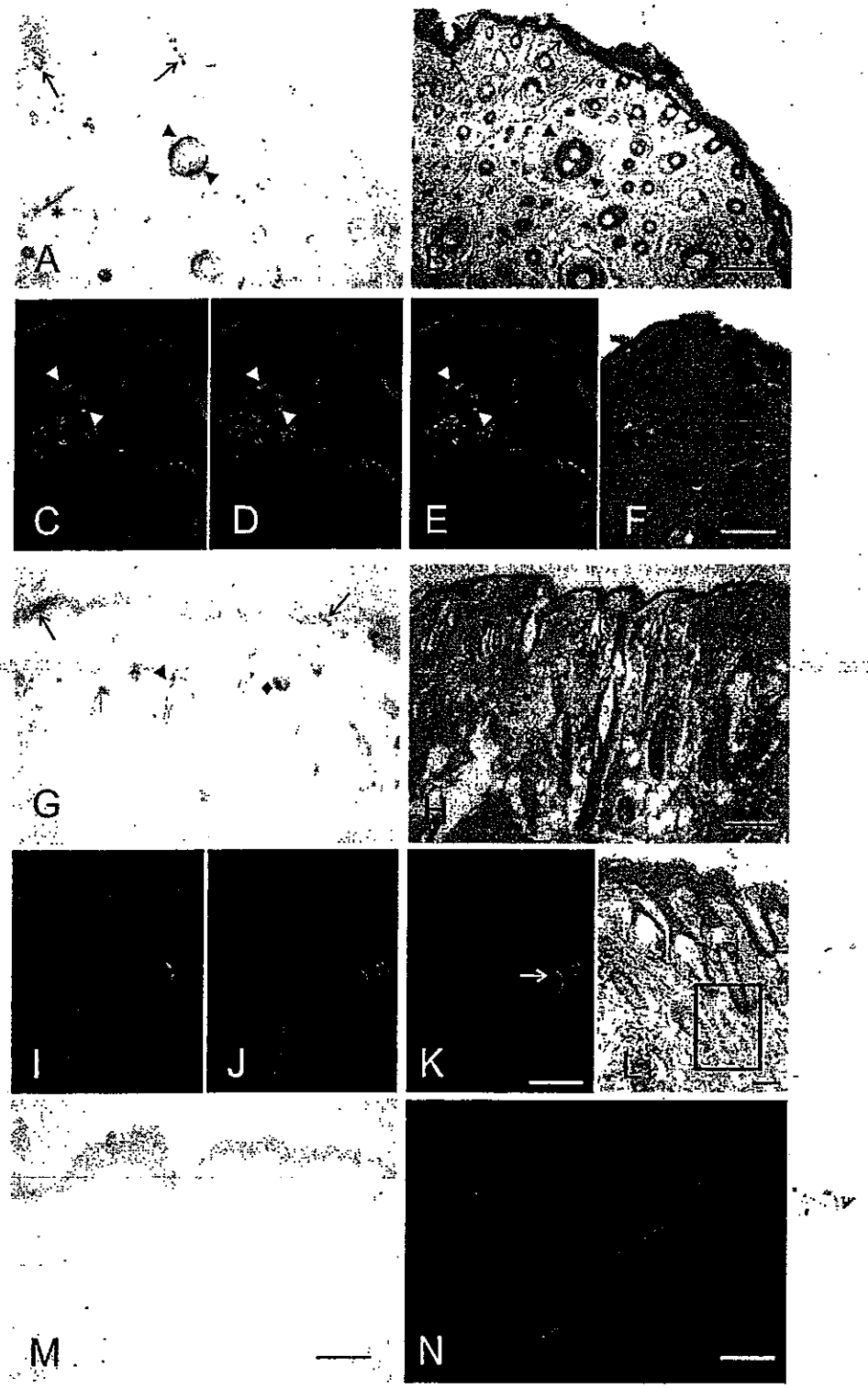

Figure 2. Location of PrP^{Sc} within the Skin of Hamsters Orally Infected with Scrapie

(A–H) Topographical localisation of PrP^{Sc} in sections of skin samples from the snout (A and B) and the forelimb (G and H); (A and G) PET blots, (B and H) H&E staining. PrP^{Sc} was detected in free nerve endings of the subepidermal plexus on the border of the epidermis to the dermis ([A], [B], [G], and [H], arrows), in fibres of the subepidermal, the deep cutaneous and the subcutaneous plexus, in circular and longitudinal fibres of the follicular neural network of the hair ([A], [B], and [G], arrowheads), in the hair follicle isthmus ([G]; rhombus), and in small intradermal striated fibres of mimic muscles ([A and B], asterisks).

(C–F) Visualisation of PrP^{Sc} and nerve fibres in the neural network of hair follicles by fluorescence microscopy (skin sample from the abdomen). Co-localisation of PrP^{Sc} (C) with nerve fibres labelled by using an anti–S-100 protein antibody against Schwann cells (D). (E) Merged figure from micrographs (C and D). (F) Adjacent section to (C), stained with H&E.

(I–K) Visualisation of PrP^{Sc} and nerve fibres in the cutaneous plexus by fluorescence microscopy (skin sample form the snout). Co-localisation of PrP^{Sc} (I) with nerve fibres labelled by using the anti-neurofilament antibody SMI 31 (J). (K) Merged figure from micrographs (I and J).
(L) Adjacent section to (I), stained with H&E. The box indicates the region used for the immunofluorescence stainings in (I–K).
(M and N) Control skin samples from the forelimb of a hamster perorally mock-challenged with normal hamster brain homogenate; PET blot (M) and fluorescence microscopy for PrP and neurofilament (N).
Scale bars = 200 μm for (B, F, H, and M), 50 μm for (K and L), and 25μm for (N). Same scale bars as displayed in (B), (F), (H), and (K) apply to (A), (C–E), (G), and (I and J), respectively.
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antibodies binding to neurofilament or Schwann cells [20,21]. By using an anti–S-100 protein antibody detecting Schwann cells, a co-localisation of PrP^{Sc} with nerve fibres of the follicular neuronal network of hairs was observed (Figure 2C–2E, arrowheads). By antibody-labelling of neurofilament, nerve fibres of the cutaneous plexus were found to display a co-localisation with PrP^{Sc} (Figure 2I–2K; for topological orientation, see Figure 2L). Occasionally, we observed PrP^{Sc} deposition in nerve-like structures of the subepidermal plexus showing no immunostaining for neurofilament (not shown). This may have resulted from infection of nerve fibres that do not contain neurofilament [20], or, alternatively, from PrP^{Sc} deposition in Schwann cells rather than in the neurite itself [22]. Control skin samples from the forelimb of a hamster perorally mock-challenged with normal hamster brain homogenate were found negative for PrP^{Sc} by PET blotting and fluorescence microscopy (Figure 2M and 2N).

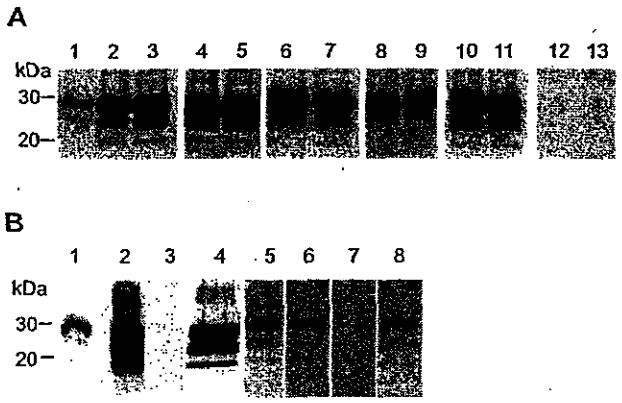

Figure 3. PrP^{Sc} Routing to the Skin and to Components of the Lymphoreticular System of Hamsters Challenged via Different Routes with 263K Scrapie Agent

(A) Western blot detection of PrP27–30, the protease-resistant core of PrP^{Sc}, in skin specimens from terminally ill scrapie hamsters. Lanes 1, 2, and 3: skin samples from orally mock-infected control hamsters, spiked before extraction with 1×10^{-6} g, 5×10^{-6} g, or 1×10^{-5} g of brain homogenate from terminally ill 263K hamsters. Lanes 4 and 5: skin samples from hindlimbs and forelimbs of hamsters orally infected with scrapie brain homogenate. Lanes 6 and 7: skin samples from hindlimbs and forelimbs of hamsters intracerebrally infected with scrapie brain homogenate. Lanes 8 and 9: skin samples from hindlimbs and forelimbs of hamsters infected by implantation of s.w. contaminated with scrapie agent. Lanes 10 and 11: skin samples from hindlimbs and forelimbs of hamsters infected peripherally by f.p. inoculation of scrapie brain homogenate. Lanes 12 and 13: skin samples from hindlimbs and forelimbs of hamsters orally mock-infected with normal brain homogenate. Amounts of tissue represented in lanes: 1, 53mg; 2, 58 mg; 3, 68 mg; 4, 68 mg; 5, 75 mg; 6, 78 mg; 7, 64 mg; 8, 69 mg; 9, 60 mg; 10, 62 mg; 11, 73 mg; 12, 61 mg; 13, 58 mg.
(B) Western blot detection of PrP27–30 in spleens and selected lymph nodes from terminally ill scrapie hamsters. Lanes 1 and 5: proteinase K-digested brain homogenate from terminally ill scrapie hamsters, containing 1×10^{-7} g brain tissue. Lanes 2–4: spleen samples from p.o.- (2), s.w.-, (3) and i.c.-infected (4) hamsters. Lanes 6–8: mesenteric lymph node samples from p.o.- (6), s.w.-, (7) and i.c.-infected (8) hamsters. Amounts of tissue represented in lanes: 2, 40 mg; 3, 45 mg; 4, 41 mg; 6, 6 mg; 7, 8 mg; 8, 6 mg.
doi:10.1371/journal.ppat.0030066.g003

Accumulation of PrP^{Sc} in the Skin Shows No Dependence on the Route of Infection and Lymphotropic Spread

In order to examine whether accumulation of PrP^{Sc} in the skin depends on the mode of infection and spreading pathways other than the nervous system, skin specimens from the forelimb and hindlimb of terminally ill hamsters challenged with 263K scrapie agent by different routes were analyzed using the same analytical approach as described above for the time-course study. The following modes of inoculation were compared: oral (p.o.) infection with scrapie brain homogenate (Figure 3A, lanes 4 and 5), intracerebral (i.c.) infection with scrapie brain homogenate (Figure 3A, lanes 6 and 7), i.c. infection by implantation of steel wires (s.w.) contaminated with scrapie agent (Figure 3A, lanes 8 and 9), and peripheral foot pad (f.p.) infection with scrapie brain homogenate (Figure 3A, lanes 10 and 11). PrP27–30 could be detected in all analyzed skin specimens from hamsters at the terminal stage of scrapie independently of the route of infection. Amounts of PrP^{Sc} in skin tissue were about 5,000- to 10,000-fold lower than those found in brain, as estimated from positive controls of skin samples from orally mock-infected hamsters that were spiked with 1×10^{-6} g, 5×10^{-6} g, or 1×10^{-5} g of homogenized 263K scrapie hamster brain from terminally ill donors before extraction (Figure 3A, lanes 1–3). PrP^{Sc} was consistently absent in skin specimens from mock-infected hamsters, which served as negative controls (Figure 3A, lanes 12 and 13).

The employed routes of inoculation were found to produce substantial differences in the extent of lymphotropic spread of agent. In orally or intracerebrally challenged hamsters, spleens (Figure 3B, lanes 2 and 4), mesenterial lymph nodes (Figure 3B, lanes 6 and 8) and retropharyngeal lymph nodes (not shown) consistently showed PrP^{Sc} deposition, whereas in five out of six hamsters infected by implantation of contaminated s.w., no PrP^{Sc} could be found in the examined spleens and lymph node specimens (Figure 3B, lanes 3 and 7). Only one of the s.w.-infected animals displayed a weak Western blot signal for PrP^{Sc} in the spleen (not shown). Thus, in comparison to the i.c. or p.o. route of inoculation, lymphotropic spread was much less pronounced—or even undetectable—following infection of hamsters by i.c. implantation of 263K-contaminated s.w. However, despite this marked discrepancy, the intensity of skin-associated deposits of PrP^{Sc} found in s.w.-infected hamsters did not show significant differences from that observed for p.o.- or i.c.-challenged animals upon Western blotting (Figure 3A, lines 8 and 9 versus lines 4–7). To determine whether the route of administration of PrP^{Sc} influences the topographical distribution of disease-associated prion protein in the skin, we investigated samples from hamsters infected with 263K-

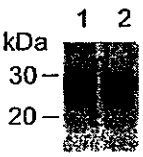

Figure 4. Western Blot Detection of PrP27–30 in Skin Specimens of Hamsters Intracerebrally Challenged with BSE-H Agent

Lanes 1 and 2: skin samples from hindlimb and forelimb of a BSE-infected hamster. Amounts of tissue represented in lanes: 1, 43 mg; 2, 58 mg.
doi:10.1371/journal.ppat.0030066.g004

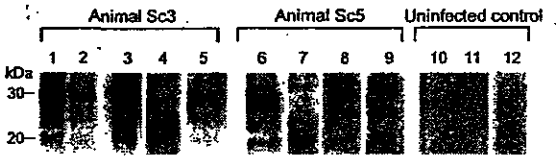

Figure 5. PrP^{Sc} in Skin Samples from Different Body Areas of Sheep Naturally Infected with Scrapie

Western blot detection of PrP27–30, the protease-resistant core of PrP^{Sc}, using the anti-PrP antibodies ICSM-18 (lanes 1–8, 10–12) and P4 (lane 9). Samples are from sheep Sc3 (lanes 1–5), sheep Sc5 (lanes 6–9), and from an uninfected control sheep. Lanes 1 and 2: different amounts of brain homogenate (0.5 mg/lane and 0.1 mg/lane of midbrain tissue, respectively) from sheep Sc3. Lane 3: tonsil sample. Lane 4: tonsil sample after deglycosylation with PNGaseF. Lane 5: skin sample from the inguinal region. Lane 6: skin sample from the perianal region (scratching area). Lane 7: skin sample from the same region as in lane 6 after deglycosylation with PNGaseF. Lane 8: skin sample from the snout. Lane 9: skin sample from the same region as in lane 8, but detected with the antibody P4. Lanes 10–12: negative controls of skin samples from different body regions of an uninfected sheep. Amounts of tissue represented in lanes: 1, 0.5 mg; 2, 0.1 mg; 3, 7 mg; 4, 2 mg; 5, 88 mg; 6, 89 mg; 7, 23 mg; 8, 90 mg; 9, 92 mg; 10, 80 mg; 11, 87 mg; 12, 94 mg.
doi:10.1371/journal.ppat.0030066.g005

contaminated s.w. at the clinical disease stage using the PET blot method. This revealed that the morphological distribution pattern of PrP^{Sc} in the skin of s.w.-infected animals was identical to that found in orally infected hamsters (not shown).

BSE-Associated PrP^{Sc} in the Skin

To test whether not only scrapie-associated PrP^{Sc} but also BSE-associated PrP^{Sc} accumulates in the skin, hamsters were intracerebrally infected with a hamster-adapted BSE (BSE-H) agent and sacrificed at the end stage of disease. All analyzed skin specimens from forelimbs and hindlimbs of five donor animals showed substantial amounts of BSE-associated PrP27–30 (Figure 4, lanes 1 and 2). Thus, following i.c. infection, the targeting of BSE-H agent to the skin, as probed by Western blotting, did not show discernible differences compared to that observed for 263K scrapie.

PrP^{Sc} in Skin Tissue of Sheep Naturally Infected with Scrapie

In a follow-up proof-of-concept study, which aimed to validate and expand our findings from the hamster experiments, five sheep naturally infected with scrapie in the field were analyzed for the presence of PrP^{Sc} in skin specimens from different body regions (head, snout, hindlimb, forelimb, perianal, axillar, and inguinal). PrP^{Sc} was visualized by Western blotting after extraction in the form of PrP27–30 from tissue samples using the anti-ovine PrP monoclonal antibodies ICSM-18 or P4. Positive specimens were found in two out of the five tested sheep: In sheep Sc3, PrP27–30 was present in a sample from the inguinal region (Figure 5, lane 5). Sheep Sc5 showed PrP27–30 in a sample from the perianal region, which was a scratching site of this animal (Figure 5, lane 6). For control purposes, a deglycosylation of a PrP27–30 extract from the same skin region of this animal was performed (Figure 5, lane 7). Furthermore, a skin sample from the snout was found positive for PrP27–30 in animal Sc5 upon detection with the ICSM-18 antibody (Figure 5, lane 8) and the anti-PrP antibody P4 (Figure 5, lane 9). All other analyzed skin specimens of the five sheep did not show specific Western blot signals for PrP27–30. Specimens from the same skin regions of four uninfected sheep served as controls and consistently produced negative results (Figure 5, lanes 10–12).

Discussion

In this study we have shown that the skin provides a reservoir for PrP^{Sc}, the biochemical marker of prion infectivity, in five different hamster TSE models, independently of whether the animals were challenged with scrapie via the p.o., i.c., or f.p. route, cerebral implantation of scrapie-contaminated s.w., or i.c. inoculation of a hamster-adapted BSE agent. Furthermore, PrP^{Sc} could be demonstrated for the first time in skin specimens from sheep naturally infected with scrapie, though in a limited number of sites investigated and at low amounts. In a time-course study using hamsters fed with scrapie agent, we were able to detect PrP^{Sc} in the skin before the onset of clinical symptoms, but the bulk of skin-associated PrP^{Sc} accumulated in the clinical phase of the disease. From our Western blot findings, the final concentration of PrP^{Sc} in the skin of hamsters seems to be approximately 5,000–10,000 times lower than that found in the brain. This would correspond to an infectivity titre of $\sim 1 \times 10^5$ to 2×10^5 50% i.c. infective doses ($ID_{50i.c.}$) per gram of skin tissue. A similar infectivity titre was previously estimated from Western blot findings for skeletal muscle tissue of clinically ill hamsters perorally challenged with 263K scrapie [23].

Pathophysiology of PrP^{Sc} Deposition in the Skin

Our immunohistochemical and PET blot studies, performed on skin specimens from hamsters orally infected with scrapie in order to elucidate the topographical location of PrP^{Sc} in the skin, revealed PrP^{Sc} in small nerve fibres within the dermis but not in keratinocytes. Keratinocytes have been shown to express PrP^{C} [24,25], and it remains to be established in future studies why this cell type—other than, for example, myocytes—does not support a detectable formation of PrP^{Sc}. Irrespective of this uncertainty, the topographical dermal location of PrP^{Sc}, which is essentially restricted to neural structures together with the late occurrence of the PrP^{Sc} in the skin, point to an invasion via centrifugal spread of infection along peripheral nerves. The time-course of PrP^{Sc} accumulation in the skin, and the putative neural spreading pathways used by prions to target this organ, are strongly reminiscent of what has been previously observed for muscle tissue in the same animal model [11]. In order to further examine whether pathways other than the nervous system may be involved in the spread

of prions to the skin, a group of hamsters was intracerebrally infected by implantation of s.w. contaminated with scrapie agent. In this experimental paradigm, lymphotropic spread of scrapie agent through the body, as evidenced by testing the spleen and selected lymph nodes for PrP^{Sc}, was not detectable in five out of six animals. Only in the spleen of one hamster from the s.w. group was a weak signal for PrP^{Sc} found. This is indicative of a splenic infection, which occurred only relatively late in the incubation period, possibly via the peripheral nervous system. Despite the striking absence of detectable lymphotropic spread in s.w.-infected hamsters, these animals produced a practically indistinguishable skin-associated accumulation of PrP^{Sc} from that observed for p.o.- or i.c.-challenged hamsters. Thus, propagation of infection to the skin did not show a crucial dependence on lymphotropic spreading pathways. Whether a blood-borne dissemination of agent contributes to the PrP^{Sc} contamination of skin additonally to neurally mediated invasion remains to be established.

Skin-Associated PrP^{Sc} Deposition in Sheep Naturally Infected with Scrapie

The Western blot examination of skin specimens from five sheep clinically affected with natural scrapie in the field revealed the presence of PrP^{Sc} in a sample from the inguinal region of one animal, and in two samples from the snout and perianal region of another sheep. The perianal region was a scratching site of this animal but did not show macroscopically visible skin alterations. Although the exact anatomical location of skin-associated PrP^{Sc} in ovine scrapie remains to be determined, our findings clearly demonstrate that the skin of sheep naturally infeceted with scrapie can provide a reservoir for prions at least at late stages of incubation. The results with skin samples from scrapie-infected sheep are in good accordance with the Western blot findings in hamsters, and the anatomical organisation of hamster and sheep skin shows considerable similarities. However, because of possible anatomical differences, histological findings in hamsters cannot be extended directly to sheep without further immunohistochemical and/or PET blot examinations in ovines. Also, for a more precise assessment of potential risks possibly emanating from prions in the skin of scrapie-infected sheep, multiple tissue sites and larger numbers of animals, including those pre-clinically incubating the disease, and random case control studies are required.

Our findings raise the question of whether prions present in specific components of the skin such as peripheral nerve fibres may be involved in the natural transmission of scrapie. Dissemination of infectivity from the skin into the environment could theoretically take place at skin lesions such as scrapie-induced chafing sites and other wounds or ulcers, and vectors found to be able to harbour infectivity (e.g., mites [26] or larvae and pupae [27]) may possibly take up prions from the skin of affected sheep. In this context, it has to be noted that inflammatory processes [25,28] may enhance the load of prions in infected skin regions, and that an increase of cellular prion protein expression was observed in keratinocytes of human patients with inflammatory skin diseases [24]. Furthermore, practises of sheep shearing may account for transmission of scrapie from reservoirs in the skin. Shearing causes skin wounds in up to a third of the sheared animals, and the skin may not only provide a reservoir of prions but also an efficient portal of entry for scrapie agent into the body [29,30]. However, previous studies on the presence of infectivity in which various tissues from scrapie-infected sheep or goats were tested did not point to the skin as a highly relevant reservoir of prions in small ruminants [31]. Furthermore, a substantial body of evidence suggests that many, if not the majority of cases of ovine scrapie, are caused by peroral uptake of TSE agents and subsequent invasion of the organism via the alimentary tract [32–35]. Contaminated placenta [31,36], saliva (as recently reported in the context of CWD) [37], or possibly feces or urine [38] appear to provide more relevant candidate sources of infection than the skin in the horizontal or vertical transmission of scrapie. Thus, whether and to what degree the skin plays a role in the spread of contagious scrapie in the field, and whether BSE-infected cattle, CWD-infected deer or vCJD-infected humans may also accumulate PrP^{Sc} in the skin, remains to be established in future studies.

Materials and Methods

Experiments in laboratory animals. All animal experiments were performed in accordance with European and German legal and ethical regulations and approved by the responsible review boards and authorities.

p.o. infection with scrapie brain homogenate. Outbred Syrian hamsters were fed individual food pellets doused with 100 μl of a 10% (w/v) hamster brain homogenate (corresponding to 10 mg of tissue) from terminally ill 263K scrapie–infected donors or uninfected controls as previously described [11]. Scrapie-infected animals were humanely sacrificed by exposure to CO_2 at 70 dpi ($n = 5$), 100 dpi ($n = 5$), and 130 dpi ($n = 5$), at the onset of at least two clinical signs for scrapie such as tremor of head or whole body, incoordination of gait, or difficulty in rising up from a supine position ($n = 5$; 138 dpi, 138 dpi, 139 dpi, 142 dpi, and 146 dpi), and at the terminal stage of disease ($n = 5$; 157 dpi, 158 dpi, 165 dpi, 169 dpi, and 171 dpi). Control animals, i.e., mock-challenged age-matched hamsters (three for each time-point), were sacrificed at corresponding time points.

i.c. infection with scrapie brain homogenate. Five outbred Syrian hamsters were intracerebrally infected with 50 μl aliquots of 1% (w/v) hamster brain homogenates in Tris-buffered saline ([TBS] 10 mM Tris HCl, 133 mM NaCl [pH 7.4]) from terminally ill donors challenged with 263K or BSE-H agent, or from uninfected control hamsters. The BSE-H agent was isolated in our laboratory after one passage of BSE agent from cattle in mice and subsequent transmission to hamsters [39]. The recipients ($n = 5$ for 263K, $n = 6$ for BSE-H) were humanely sacrificed at the end stage of clinical disease at the following dpi: 263K—83, 84, 87, 90, and 93 dpi; BSE-H —296, 296, 300, 310, 317, and 324 dpi.

i.c. infection by implantation of s.w. contaminated with scrapie agent. s.w. contaminated with 10% (w/v) brain homogenate from 263K scrapie hamsters were prepared as previously described [40] and intracerebrally implanted under anesthesia into reporter animals using a stereotaxic apparatus (K. Lemmer, M. Mielke, G. Pauli, and M. Beekes, unpublished data). Animals ($n = 4$) were humanely sacrificed at the terminal stage of scrapie at the following dpi: 91, 91, 91, and 106.

f.p. infections with scrapie brain homogenate. Outbred Syrian hamsters were challenged peripherally via the f.p. as described elsewhere [41] by inoculation of 50 μl of 1% (w/v) hamster brain homogenates in TBS from terminally ill 263K scrapie hamsters. Sacrification was performed humanely at the terminal stage of disease at the following dpi ($n = 4$): 117, 117, 131, and 147.

Sample preparations. *Preparation of hamster tissue samples for Western blot analyses.* After sacrifice, skin specimens from different regions of the body were dissected and the fur was removed by shaving. The skin specimens consisted of epidermis, dermis, and subcutis. All samples were cut into small pieces and stored at −80 °C until examination. The mass of the skin samples used for Western blot analyses ranged from approximately 40 to 100 mg. Instruments used for the preparation of samples were carefully cleaned after removal and processing of each specimen in order to avoid cross-contamination. Skin samples from the hindlimb, forelimb, head, back, and abdomen were taken from p.o.- and s.w.-infected animals. From the hamsters i.c.- or f.p.-inoculated with 263K scrapie and the animals i.c.-

Table 1. Scrapie-Infected Sheep Tested for PrP^{Sc} in the Skin

Animal	Genotype[a]	Age[b]	PrP^{Sc} in Brainstem	PrP^{Sc} in Cerebellum
Sc1	ARQ/ARQ	> 4 years	Yes	Yes
Sc2	ARQ/ARQ	> 4 years	Yes	Yes
Sc3	ARQ/ARQ	> 4 years	Yes	Yes
Sc4	AHQ/ARQ	> 4 years	Yes	Yes
Sc5	ARQ/ARQ	> 4 years	Yes	Yes

[a]The genotype is specified for the polymorphic codons 136, 154, and 171 of the ovine prion protein gene.
[b]Age was determined by the dental status of the animals.
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inoculated with BSE-H agent, skin samples were dissected from the forelimb and hindlimb.

Preparation of hamster tissue samples for PET blot and immunohistochemical analyses. For morphological PET blot analyses on the distribution and location of PrP^{Sc} in the skin, specimens from the head, the snout, the abdomen, and the foreleg were collected. Samples were taken from hamsters that developed terminal symptoms of scrapie after peroral or s.w. infection and from age-matched perorally mock-infected controls sacrificed at corresponding time-points. The specimens were either immediately snap frozen or fixed in paraformaldehyde (4% [w/v] in PBS) or Carnoy's solution containing 60% isopropanol, 30% dichloromethane, and 10% acetic acid for up to 48 h and embedded in paraffin. Sections were placed on glass slides and a subset was stained with haematoxylin and eosin (H&E).

Sample preparation from sheep naturally infected with scrapie. Skin samples from field cases of ovine scrapie were collected during autopsies of cohort animals that were eradicated after identification of an index scrapie case in the respective flock. The samples analysed in this study originated from five scrapie cases (Table 1, Sc1–Sc5) and four scrapie-free control sheep. All scrapie cases were older than 4 years as determined by their dental status and showed loss of wool due to scrubbing their coat. They also nibbled off their fleece on the legs over the carpal and tarsal joints where the straight coat becomes wooly. In all scrapie-infected animals, a marked weight loss was present. The diagnosis of scrapie was confirmed according to Office International des Epizooties standards by histopathology and by immunohistochemical detection of PrP^{Sc} in the brain stem and cerebellum. Apart from animal Sc4, PrP^{Sc} could also be detected in the tonsils from all scrapie sheep (not shown). At the polymorphic codons 136, 154, and 171 of the prion protein gene, four of them were ARQ/ARQ and one animal (Sc4) was AHQ/ARQ. According to the National Scrapie Plan for Great Britain [42], the genotypes ARQ/ARQ and AHQ/ARQ belong to risk group 3, which refers to "sheep that genetically have little resistance to scrapie and will need careful selection when used for further breeding". It has to be noted, however, that ARQ/ARQ and AHQ/ARQ do not provide the most prevelant genotypes in scrapie-affected sheep.

Western blot examinations. For Western blot analyses, samples were washed three times in TBS (10 mM Tris HCl, 133 mM NaCl [pH 7.4]) and incubated in a rocking device at 37 °C for 4 h in 900 µl of TBS containing 2 mM $CaCl_2$ and 0.25% (w/v) collagenase A (Roche, http://www.roche.com). For positive controls, skin tissue from orally mock-infected control donors was spiked by adding 5 µl of a 0.1 % (w/v) 263K scrapie hamster brain homogenate (i.e., 5 µg of brain tissue) from i.c.-infected donors containing approximately 0.5 ng of PrP^{Sc} [3] prior to collagenase digestion. After ultrasonification to disrupt remaining tissue structures, the samples were centrifuged for 3 min at 500*g*. The supernatant was carefully transferred to a new cup, whereas the pellet consisting of cell debris and the rest of the fur was removed. Subsequently, PrP^{Sc} was extracted in the form of PrP27–30 from the tissue homogenates following a previously published protocol [23]. Proteinase K–digested homogenate from 263K scrapie hamster brains, used as a PrP27–30 reference in the Western blotting analyses, was prepared as outlined previously [23]. For deglycosylation [23], extracted pellets were dissolved in 20 µl of a. bidest and one fourth of the aliquots was digested using PNGase F (New England Biolabs, http://www.neb.com) according to the instructions of the manufacturer prior to Western blotting. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses of samples from hamsters were performed as described elsewhere [23]. Western blot testing for PrP^{Sc} in skin samples from scrapie-infected sheep was similarly performed using the primary antibodies ICSM-18 and P4 to label the ovine prion protein. PrP signals were visualized on an X-OMAT AR (Kodak, http://www.kodak.com) film. Films were exposed for 5–30 min.

PET blot examinations. The PET blot detection of proteinase K–resistant PrP deposits in sections of skin samples was performed on paraformaldehyde- and Carnoy-fixed specimens. For PET blot examinations of skin tissue, modifications of the original protocol [43] were necessary to remove connective tissue. After prewetting blots with TBST (10 mM TrisHCl [pH 7.8], 100 mM NaCl, 0.05 % [w/v] Tween 20), sections were digested with 1.5 mg/ml collagenase A (Roche) in a buffer containing 10 mM TrisHCl (pH 7.8), 100 mM NaCl, 100 mM $CaCl_2$, and 0.1% (w/v) Brij 35 for 30 min at 60 °C, followed by digestion using 250 µg/ml proteinase K (Roche) in PK digestion buffer (10 mM TrisHCl [pH 7.8], 100 mM NaCl, 0.1% Brij 35) for 8 h at 55 °C. After this step, the membrane-attached proteins were fixated to the membrane. The proteins on the membranes were denatured with 3 M guanidine isothiocyanate in 10 mM TrisHCl (pH 7.8) for 20 min. Immunodetection was performed after preincubation in blocking solution (0.2% [w/v] casein in TBST) for 30 min. The monoclonal antibody 3F4 ([44]; diluted 1:3,000) was used as primary antibody and an alkaline phosphatase-coupled rabbit anti-mouse antibody (Dako, http://www.dako.com) at a dilution of 1:500 as secondary antibody. Visualization of antibody binding was achieved by using NBT/BCIP. Blots were examined using an Olympus dissecting microscope.

Immunohistochemical examinations. Frozen tissue sections as well as Carnoy-fixed sections of about 4 µm were postfixed in paraformaldehyde (4% [w/v] in PBS) for 1 h and washed in tap water to avoid pigment deposition. Antigen retrieval was done using 4 M guanidine hydrochloride for 25 min and by microwaving five times for 3 min at 700 watts in 1 M citric acid at pH 6.0. After blocking with 0.2% casein in TBST, the primary antibody 3F4 (diluted 1:200) was used for PrP detection. Antibodies against S-100 protein (rabbit polyclonal, 1:200 in PBS; Dako,) and neurofilament (SMI31 mouse monoclonal IgG1, 1:20,000 in PBS; Sternberger Monoclonals, http://www.crpinc.com) were applied to detect small nerve fibres. As secondary antibody, a HRP-conjugated goat anti-mouse IgG1 antibody (1:400 in PBS; Dianova, http://www.dianova.de) was used for detecting the antibody SMI31. The S-100 antibody was detected using a polyvalent HRP-conjugated goat anti-rabbit antibody (EnVision, Dako). For 3F4 doublestaining, we used a biotinylated goat anti-mouse IgG2a antibody (1:400 in PBS; Dianova) or a goat anti-mouse IgG antibody without restriction to Ig-subclasses (1:100; Dako). Visualization of the different epitopes was performed using Cy2-conjugated streptavidine (green fluorescence, 1:500; Dianova) and a Cy3-conjugated goat anti-HRP antibody (red fluorescence, 1:100; Dianova). Slides were examined on an Olympus fluorescent microscope using analysis software, and Adobe Photoshop software was used for picture processing.

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Author contributions. AT and MB conceived and designed the experiments. AT, WSS, and AW performed the experiments. AT, WSS, AW, and MB analyzed the data. AT, WSS, AW, WW, BB, CK, and KL contributed reagents/materials/analysis tools. AT, WSS and MB wrote the paper.

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No. 26

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研究報告の概要

○73歳受血者における変異型クロイツフェルト・ヤコブ病の非定型症状
73歳の受血者に生前に特定された変異型クロイツフェルト・ヤコブ病(vCJD)の非典型的症状の報告である。当該患者は、1997年12月の外科手術後に輸血を受けた。血液製剤の追跡により、供血から11ヵ月後に神経病理学的に確定されたvCJDを発現した供血者由来の白血球非除去赤血球製剤一製剤が特定された。この患者は、輸血から9年後に専門家の診察を受けるためNational Prion Clinicへ紹介された。輸血から6年後、受血者は変動する疲労および集中困難を訴えた。この時点での神経学的検査及び脳MRI(T1/T2 weighted/DWI)は正常であった。この6ヵ月後に、平衡失調と認知の低下をともなう進行性症状が発現した。神経学的症状発現から2ヵ月後の検査では、認知障害、失行、視空間障害が示されたが、運動、感覚、歩行検査は正常であった。6週間後、振せん、手指の作業能力障害、四肢の失調を伴う認知障害が認められた。血清学的検査は正常であった。MRI(T1/T2 weighted/FLAIR/DWI)では、vCJDの所見と一致する、視床背側核全体の顕著な信号変化が示された。PRNP遺伝子型検査では、変異はなく、コドン129のメチオニンはホモ接合体であることが判明した。vCJDの長期潜伏期間と無症候性キャリア状態の可能性があることは、重大な公衆衛生問題を提示する。本症例は、汚染血液製剤の受血者が遭遇した重大な危険性と、専門家のモニタリングの必要性を浮き彫りにした。

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
輸血によって変異型クロイツフェルト・ヤコブ病(vCJD)に感染したと疑われる73歳の受血者が生前に診断されたvCJDの非典型的症状の報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

39

Epidemiology, Risk Assessment and Transmission

P04.49

Case Report of Variant Creutzfeldt-Jakob Disease in a Macaque after Blood Transfusion

Lescoutra-Etchegaray, N[1]; Ruchoux, MM[1]; Correia, E[1]; Joli, A[1]; Freire, S[1]; Lasmezas, CI[2]; Deslys, JP[1]; Comoy, E[1]
[1]CEA/DSV/IMETI/SEPIA, France; [2]Scripps Florida, USA

A fourth human case of probable transmission of vCJD through transfusion has now been reported but a number of features affecting transfusion-related infection remain imprecise, including infectious dose, length of incubation period and critical infectious window of blood donors.

We report here the first case of experimental transmission of vCJD in primates by blood transfusion. Experimental infection of Cynomolgus macaque has been demonstrated to be a sensitive model for the investigation of human prion diseases, inducing similar distribution of infectivity in peripheral lymphoid tissues and equivalent brain pathology. In our study, transfusion was performed with 40 ml of whole blood drawn from a vCJD-infected macaque at the terminal stage of the disease. Clinical symptoms of vCJD appeared in the recipient animal after five years of incubation. The total amount of infectivity in the transfused blood was approximately 106 fold lower than in the brain (titration still in progress). In several animals infected intravenously with brain homogenate, the presence of PrPres in serial lymph nodes biopsies and in other organs at autopsy was examined and results will be presented.

P04.50

Regional and Global Apparent Diffusion Coefficient in Inherited Prion Disease: Correlation with Disease Severity

Wroe, S[1]; Hyare, H[2]; Thornton, JS[2]; Yousry, T[2]; Siddique, D[1]; Webb, T[1]; Collinge, J[1]
[1]National Hospital for Neurology and Neurosurgery, UK; [2]National Hospital for Neurology and Neurosurgery, UK

Cerebral diffusion weighted MR imaging (DWI) has recently emerged as the most sensitive sequence for the diagnosis of prion diseases with reports of apparent diffusion coefficient (ADC) changes in specific anatomical regions. This study examined regional and global changes in cerebral ADC in a large cohort of patients with inherited prion disease, and by correlation with clinical indices investigated their potential as biomarkers of disease progression. Twenty five patients (14 female, mean age 45.2 years, range 32-69 years) with inherited prion disease underwent echo-planar DWI (b1000, TE101ms) and conventional T2W and FLAIR imaging at 1.5T. Mean region-of-interest (ROI) ADCs for the head of caudate, putamen and pulvinar nuclei were determined bilaterally and volume-normalised whole-brain ADC histograms computed following tissue segmentation. Clinical assessment included the Mini Mental State Examination (MMSE), Alzheimer's Disease Assessment Scale (ADAS-COG), Clinician's Dementia Rating (CDR) and the Rating of Global Severity (GS). The Spearman rank correlation coefficient was calculated for each of the ROI mean ADCs, the whole-brain mean and median ADCs, and histogram peak height and peak position, versus clinical score, with $p<0.01$ considered significant. For the whole-brain measures, significant correlations were with MMSE: whole-brain mean ADC vs. MMSE: $r=-0.53$, $p=0.008$, whole-brain ADC histogram peak height vs. MMSE: $r=0.51$, $p=0.01$ and median whole-brain ADC vs. MMSE: $r=-0.52$, $p=0.009$). Of the ROIs investigated, bilateral pulvinar mean ROI ADCs alone correlated significantly with each of the clinical scores, the most significant correlations being between the pulvinar mean ADC and ADAS-COG: right: $r=0.77$, $p<0.001$ (Fig 3) and left: $r=0.56$, $p=0.009$ (Fig 4). No pathological signal changes were detected on conventional MR imaging. Both anatomically specific and whole-brain ADC metrics correlated with disease severity. Conventional MR imaging, including visual assessment of DWI, was relatively insensitive to cerebral pathology in this patient group. Despite this, we have shown that quantification of cerebral ADC provides both regional and global measures that correlate with clinical neurological status and therefore show promise as quantitative pathological biomarkers in inherited prion disease.

P04.51

Atypical Presentation of Variant Creutzfeldt-Jakob Disease in a 73 Year Old Blood Transfusion Recipient

Wroe, S[1]; Pal, S[1]; Webb, T[1]; Alner, K[2]; Hewitt, P[3]; Brander, S[4]; Wadsworth, JD[5]; Collinge, J[1]
[1]National Hospital for Neurology and Neurosurgery, National Prion Clinic, UK; [2]National Hospital for Neurology and Neurosurgery, Department of Neuropsychology, UK; [3]Health Protection Agency, UK; [4]National Hospital for Neurology and Neurosurgery, Department of Neuropathology, UK; [5]Institute of Neurology, UCL, UK

We report atypical presentation of variant Creutzfeldt-Jakob Disease (vCJD) identified ante-mortem in a 73 year-old recipient of blood products. This patient was transfused following orthopaedic surgery in December 1997. Tracing of blood products identified a single unit of non-leucodepleted red cells from an individual who developed neuropathologically confirmed vCJD eleven months after donation. Nine years post transfusion, this individual was referred to the National Prion Clinic for specialist investigation. Six years post transfusion the recipient complained of fluctuating fatigue and impaired concentration. At this time neurological examination and MRI brain (T1/T2 weighted/DWI) were normal. Progressive symptoms emerged six months later with imbalance and deteriorating cognition. Examination two months after onset of neurological symptoms demonstrated cognitive deficits, dyspraxia or visuospatial dysfunction and normal motor, sensory and gait examination. Six weeks later cognitive impairment was identified alongside tremulousness, impaired manual dexterity and limb ataxia. Serological investigations were normal. MRI (T1/T2 weighted/FLAIR/DWI) demonstrated prominent signal change throughout the dorsal thalamus, consistent with vCJD. PRNP genotyping revealed no mutations and homozygosity for methionine at codon 129. The prolonged incubation period of vCJD and possibility of asymptomatic carrier states pose major public health concerns. This case highlights the significant risk encountered by recipients of contaminated blood products and the necessity for their specialist monitoring.

P04.52

Quantification of Brain Atrophy Rates in CJD Using Volumetric MRI

Wroe, S[1]; Macfarlane, RG[1]; Scahill, R[2]; Yousry, TA[3]; Collinge, J[1]
[1]Dept of Neurodegenerative Disease, Institute of Neurology, MRC Prion Unit, UK; [2]Institute of Neurology, UK Dementia Research group, UK; [3]National Hospital for Neurology and Neurosurgery, UK

Brain atrophy has been described in all forms of human prion disease. Most reports are from visual inspection and subjective impression by radiologists who may or may not be blinded to diagnosis. This study applied quantitative measures of the rate of brain atrophy to patients with prion disease. Subjects were patients with any form of human prion disease. A control group of 12 age and sex matched normal subjects was also identified. MRIs were performed at 0, 1, 2, 4 and 6 months from trial enrolment and 3 monthly thereafter. All subjects and controls had T1 weighted volumetric scans acquired at 1.5T. Image processing was performed using MIDAS (Medical Image Display and Analysis Software) with a protocol for whole brain and cerebellar segmentation. Each scan was bias corrected to even out any intensity inhomogeneity and then underwent segmentation, registration and brain boundary shift integral (BSI) calculation from which annual rates of whole brain and cerebellar atrophy were calculated. All image processing was done in a blinded manner. 217 MRI scans were performed in 44 patients. Each patient had between 1 and 19 scans each (mean 4.50, median 3). Seventy nine scans were discarded, mainly due to movement artefact, leaving 138 scans remained in 31 patients. The mean interval between first and last scans in each patient was 1.24 years. One variant CJD, two sporadic CJD and 28 inherited prion disease patients (the majority with 6-OPRI or P102L PRNP mutations) were studied. Control patients were identified from a familial Alzheimer's trial and had two scans each, an average of 1.78 years apart (range1.05-3.15). This study showed that rates of brain atrophy and cerebellar atrophy were significantly higher in symptomatic CJD patients than in control patients. Mean whole brain atrophy rates were 1.3%/year higher than controls (whose mean rate was 0.17%/year). Whole brains did not atrophy at a significantly different rate to cerebelli. Atrophy rates were not significantly different according to presence of symptoms, sex, disease type or inherited disease type in this set of patients. No asymptomatic patients were deemed to have become symptomatic during the study. This is the first description of quantification of rates of brain atrophy in prion disease. Rate of brain atrophy may be a useful prognostic indicator or outcome measure in future clinical trials in human prion disease.

医薬品 研究報告 調査報告書

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研究報告の概要

○フォアグラのアミロイド生成の可能性

ヒトの脳及び全身性アミロイドーシスならびにプリオンに関連した海綿状脳症は、後天的、遺伝的なタンパク質折りたたみ構造の異常である。正常の場合、可溶性タンパク質やペプチドは線維性凝集体に変換される。これは核形成依存性プロセスであり、アミロイド沈着促進因子(AEF)である同種・異種アミロイド前駆体から形成される繊維の種(seed)によって始まるか、あるいは加速すると考えられ、これらの構造的に変化した成分の経口摂取又は非経口投与により疾患が伝播するという重大な病原性を持つ。感染脳組織以外に、はっきりとしたAEFの食物源は特定されていない。本稿では、二次性(アミロイドAタンパク質;AA)アミロイドーシスを誘起したトランスジェニックマウスモデルにおいて強力なAEF作用を示す血清アミロイドA関連タンパク質からなる複屈折性コンゴレッド親和性線維状物質が、市販のアヒルまたは鴨由来フォアグラに含まれることを報告する。フォアグラから抽出したアミロイドをこのマウスに投与または摂取させると、全身性異常沈着が多数発現した。これらの実験データは、アミロイド含有食品が、感受性集団においてAAアミロイドーシスを促進させるというエビデンスを示している。以上のデータから、二次性アミロイドーシスと(おそらく)その他のアミロイドーシスが伝播性であり、プリオン関連疾患の感染性と類似する可能性があると推測される。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
フォアグラから抽出したアミロイドを二次性アミロイドーシスを誘起したトランスジェニックマウスに投与または摂取させたところ、全身性異常沈着が多数発現し、二次性アミロイドーシスとその他のアミロイドーシスが伝播性であり、プリオン関連疾患の感染性と類似する可能性があると推測されたとの報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

Amyloidogenic potential of foie gras

Alan Solomon*†, Tina Richey*, Charles L. Murphy*, Deborah T. Weiss*, Jonathan S. Wall*, Gunilla T. Westermark‡, and Per Westermark§

*Human Immunology and Cancer Program, Department of Medicine, University of Tennessee Graduate School of Medicine, Knoxville, TN 37920; ‡Division of Cell Biology, Linköping University, SE-58185 Linköping, Sweden; and §Department of Genetics and Pathology, Uppsala University, SE-75185 Uppsala, Sweden

Communicated by D. Carleton Gajdusek, Institut de Neurobiologie Alfred Fessard, Gif-sur-Yvette, France, January 30, 2007 (received for review October 10, 2006)

The human cerebral and systemic amyloidoses and prion-associated spongiform encephalopathies are acquired or inherited protein folding disorders in which normally soluble proteins or peptides are converted into fibrillar aggregates. This is a nucleation-dependent process that can be initiated or accelerated by fibril seeds formed from homologous or heterologous amyloidogenic precursors that serve as an amyloid enhancing factor (AEF) and has pathogenic significance in that disease may be transmitted by oral ingestion or parenteral administration of these conformationally altered components. Except for infected brain tissue, specific dietary sources of AEF have not been identified. Here we report that commercially available duck- or goose-derived foie gras contains birefringent congophilic fibrillar material composed of serum amyloid A-related protein that acted as a potent AEF in a transgenic murine model of secondary (amyloid A protein) amyloidosis. When such mice were injected with or fed amyloid extracted from foie gras, the animals developed extensive systemic pathological deposits. These experimental data provide evidence that an amyloid-containing food product hastened the development of amyloid protein A amyloidosis in a susceptible population. On this basis, we posit that this and perhaps other forms of amyloidosis may be transmissible, akin to the infectious nature of prion-related illnesses.

amyloid protein A amyloidosis | amyloid-enhancing factor | protein aggregation | rheumatoid arthritis | transmissibility

Amyloid protein A amyloidosis (AA) occurs in patients with rheumatoid arthritis and other chronic inflammatory diseases and results from a sustained elevation of the apolipoprotein serum amyloid A (SAA) protein produced by hepatocytes under regulation by interleukin (IL)-1, IL-6, and tumor necrosis factor (1). This acute-phase reactant is cleaved into an ≈76-residue N-terminal fragment deposited as amyloid predominately in the kidneys, liver, and spleen. The disorder also can be induced experimentally in susceptible strains of mice by inflammatory stimuli that result in an >1,000-fold increase in SAA concentration (2). Further, the lag phase of this process is greatly decreased by injecting or feeding animals extracts of amyloid-laden spleens of affected mice (2–5).

To determine whether amyloid-containing food products exhibit amyloid enhancing factor (AEF) activity, we used a more robust *in vivo* murine model of AA amyloidosis involving mice carrying the human *IL-6* (*hIL-6*) gene under control of either the murine metallothionein-1 (MT-1) (MT-1/hIL-6) or histocompatibility H2-L^d (H2/hIL-6) promoter (6). Typically, AA amyloid develops in these animals at ≈5 mo of age and is initially located predominately in the perifollicular regions of the spleen. Over the next 2–3 mo, the deposits spread rapidly into the liver parenchyma, renal glomerular and intertubular regions, cardiac muscle, tongue, and gastrointestinal tract and lead to death at ≈8–9 mo. However, by injection into 8-wk-old transgenic mice of a single 100-μg i.v. dose of an exogenous source of AA fibrils, amyloid deposits are formed within 3 wk, and severe systemic disease (akin to that found in 8-mo-old animals) occurs within 2 mo, at which time the resultant pathology is lethal (7).

AA amyloid deposits are commonly found in waterfowl, particularly Pekin ducks, in which the liver is predominately involved (8–10). This pathological alteration is noticeably increased in birds subjected to stressful environmental conditions as well as to the forced feeding that is used to produce foie gras (8). This culinary product, derived from massively enlarged fatty livers results from gorging young ducks or geese up to three times daily over a 4-wk period with corn-based feed.

We now report the results of our studies that have shown that AA-containing fibrils extracted from duck or goose foie gras have potent AEF activity when administered by i.v. injection or gavage into our IL-6 transgenic mice.

Results and Discussion

We analyzed several commercial sources of foie gras histochemically and found amyloid to be present. Microscopic examination of hematoxylin/eosin- and Congo red-stained sections cut from formalin-fixed, paraffin-embedded specimens revealed virtual replacement of the normal hepatic parenchyma by fat; additionally, green birefringent congophilic areas in residual vasculature were noted by polarizing microscopy (Fig. 1 *a* and *b*). Further, these deposits were immunostained by a specific anti-AA antiserum (Fig. 1*c*). Similar material was found in marketed pâtés prepared from duck or goose liver (Fig. 2).

The AA composition of the hepatic amyloid deposits was confirmed chemically through analysis of material derived from acetone-defatted specimens extracted first with 0.15 M NaCl and then distilled water. The isolates were strongly congophilic, and, when examined by transmission electron microscopy, contained fibrils with the typical ultrastructural features of amyloid; namely, ≈10-μm-thick unbranched structures (Fig. 3*a*). Electrophoresis of the water-suspended product on a SDS/polyacrylamide gel in the presence of 0.1 M DTT and 8 M urea revealed, after Coomassie blue staining, a protein band with a M_r of ~6,000, comparable to that of amyloid extracted from the spleen of a mouse with AA amyloidosis (Fig. 3*b*). After transfer to a PVDF membrane, this component was subjected to automated Edman degradation with which 14 residues identical in amino acid sequence to that of the N-terminal portion of duck SAA were detected. In a similar study of tryptic digests obtained from cleavage of this molecule after reduction and alkylation, six peptides that included 45 of the first 60 residues of duck SAA were identified by MS/MS (Fig. 3*c*) (9).

Author contributions: A.S., J.S.W., and P.W. designed research; T.R. and C.L.M. performed research; J.S.W., G.T.W., and P.W. analyzed data; A.S. and D.T.W. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

Abbreviations: AA, amyloid protein A amyloidosis; AEF, amyloid enhancing factor; IL, interleukin; SAA, serum amyloid A protein.

†To whom correspondence should be addressed at: University of Tennessee Graduate School of Medicine, 1924 Alcoa Highway, Knoxville, TN 37920. E-mail: asolomon@mc.utmck.edu.

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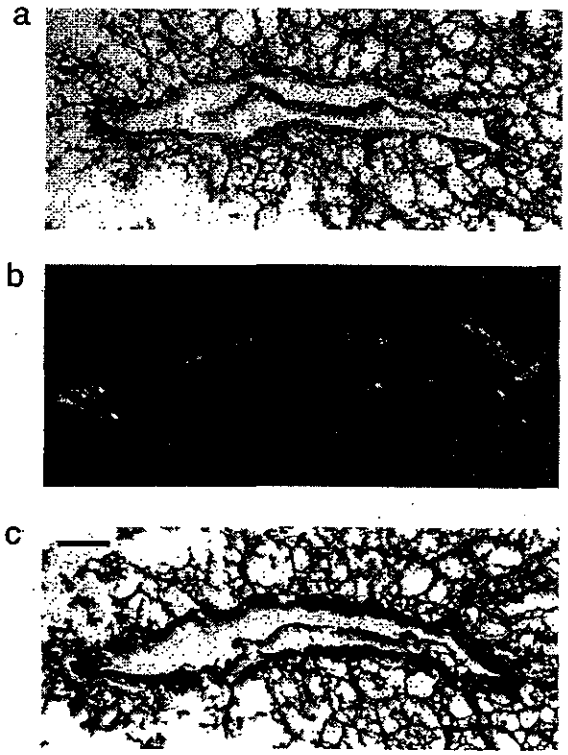

Fig. 1. AA deposition in foie gras. (a) Large venule surrounded by residual, extensively vacuolated fatty hepatic tissue (hematoxylin/eosin stain). (b) Green birefringent amyloid deposits in the blood vessel wall (Congo red stain). (c) Immunohistochemical identification of vascular AA amyloid. (Scale bar, 62 μm.)

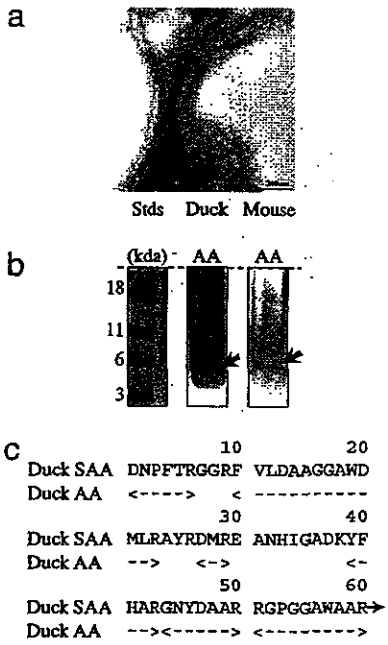

Fig. 3. Ultrastructural and chemical characterization of amyloid extracted from foie gras. (a) Fibrillar nature of proteins contained in the pellet (electron micrograph, negative uranyl acetate stain). (Scale bar, 200 nm.) (b) SDS/PAGE of congophilic components extracted from duck foie gras and the spleen of a mouse with AA amyloidosis (Coomassie blue stain). The M_r values of the standard proteins are given; arrows show the location of AA-containing protein bands. (c) Comparison of the amino acid sequence of duck foie gras AA amyloid with that of duck SAA (9). Homologous residues are indicated by dashes.

To determine whether amyloid-containing duck- or goose-derived foie gras had AEF activity, groups of up to nine MT-1/hIL-6 or H2/hIL-6 mice received tail vein injections of either 100 μg of extract suspended in 0.1 ml of PBS or the equivalent volume of PBS alone. Both sets were euthanized 8 wk later and multiple organs (liver, spleen, kidney, pancreas, heart, lung, tongue, and intestines) were obtained at necropsy for histochemical analysis. Examination by polarizing microscopy of Congo red-stained sections revealed the presence of varying amounts of amyloid deposits in one or more tissues of virtually all of the treated mice; most affected were the liver, spleen, and a lesser extent, the kidneys and pancreas (Fig. 4*a*). In contrast, control animals that received PBS had no detectable amyloid.

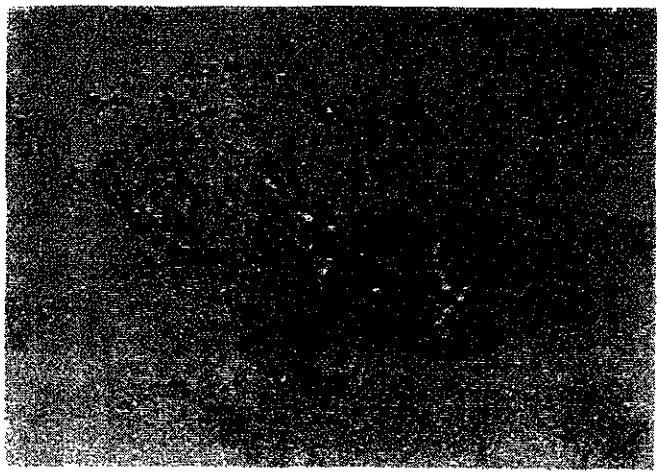

Fig. 2. Tissue fragment with amyloid in duck pâté. Congo red stain.

Similar results were obtained in the conventional murine model of AA amyloidosis in which SAA overexpression was induced by an inflammatory stimulus (4). Two groups of wild-type BALB/c mice were given two 0.5-ml s.c. injections of aqueous 1% $AgNO_3$ (days 1 and 10), and one set also were injected i.v. with 100 μg of the foie gras extract; the others (controls) received PBS only. At the time of euthanizing (day 21), 8 of 10 mice from the first group had detectable amyloid in the liver and spleen. In contrast, no amyloid was found in the control animals.

The amyloid induced by administration of fibril-containing foie gras into both the wild-type and transgenic mice was immunostained by an anti-AA antibody. Further, the deposits were AA in nature as confirmed by MS of protein extracted from the spleen of a recipient animal. MS/MS analyses of tryptic peptides generated from an HPLC-purified reduced and alkylated water pellet identified residues 19–56 of murine SAA.

AA-containing foie gras extracts also had AEF activity when administered orally to the hIL-6 transgenic animals. Five of eight mice that were gavaged for 5 consecutive days with 100 μg of material suspended in 50 μl of PBS were found 8 wk later to have amyloid deposits in virtually all organs examined, and, as in the case of animals injected i.v. with this material, this effect was most pronounced in the liver and spleen (Fig. 4*b*).

The AEF activity of foie gras was reduced, but not abolished, by cooking, as specified by the supplier. Intravenous injections into nine hIL-6 transgenic mice of 100-μg doses of extracts prepared from liver that had been heated to ≈95°C for 20 min in an oven resulted in 4+, 2+, and 1+ hepatic and/or splenic amyloid deposits in two, one, and two animals, respectively (in four cases, no amyloid was found). In contrast, when this material was dissolved in 6 M guanidine HCl, incubated at 37°C for 24 h, dialyzed against PBS, and injected into six transgenic

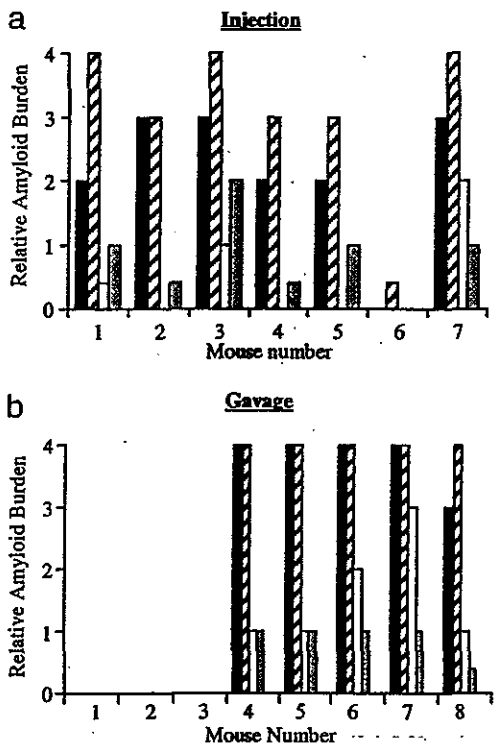

Fig. 4. AEF activity of foie gras. Hepatic and splenic amyloid deposits found in hIL-6 transgenic mice 8 wk after they were injected (*a*) or gavaged (*b*) with AA fibrils extracted from foie gras. The extent of amyloid burden in the liver (■), spleen (▨), kidney (□), and pancreas (▤) is as indicated. (Scale bars, 100 μm.)

mice, only traces of splenic amyloid were found in two mice. A summary of the results of all of the above studies is presented in Table 1 and supporting information (SI) Fig. 5.

The prevalence of AA amyloidosis in the human population is unknown. In most developed countries, sustained inflammatory processes, particularly rheumatoid and juvenile chronic arthritis (as opposed to infectious diseases), now account for the majority of such cases (11). Notably, there is a marked geographic variation in incidence among such patients, e.g., the number is relatively high in Europe compared with the United States but particularly high in parts of Papua New Guinea (12). This difference has been attributed, in part, to genetic factors, including expression of a more amyloidogenic SAA allotype (13) or other genes encoding inflammatory molecules (11). Notably, increased blood levels of SAA do not necessarily result in amyloidosis (11, 14).

Given our experimental findings, exposure to exogenous substances with AEF activity also may be an important epigenetic or environmental factor in the development of AA amyloidosis in a susceptible population. In this regard, it would seem prudent for children and adults with rheumatoid arthritis or other diseases who are at risk for this disorder to avoid foods that may be contaminated with AA fibrils (15). In addition to foie gras, meat derived from sheep (16) and seemingly healthy cattle (17) may represent other dietary sources of this material. Further, the fact that chemically heterologous fibrils can serve as AEF, as demonstrated in experimental models of AA (18–20) and AApoAII amyloidosis (21–23), suggests that it may be hazardous for individuals who are prone to develop other types of amyloid-associated disorders, e.g., Alzheimer's disease or type II diabetes, to consume such products.

Materials and Methods

Materials. Whole fresh duck and goose liver (foie gras) was purchased from three commercial venders located in the United States and France.

Mice. Mice carrying the *hIL-6* gene under control of the mouse MT-1 or histocompatibility H_2-L^d promoter were obtained from Gennaro Ciliberto and Michael Potter, respectively, and generated as described previously (6). At 4 wk the mice were weaned, and the presence of the transgene was confirmed through analysis of genomic DNA derived from tail snips. Wild-type BALB/c mice were purchased from Charles River Laboratories (Boston, MA). Animals were housed in groups of eight in a positive pressure environment with a 12-h light/dark cycle and provided filtered tap water and a standard laboratory rodent chow (Harlan Teklad, Madison, WI) ab libitum. The animals were treated in accordance with National Institutes of Health regulations under the aegis of a protocol approved by the University of Tennessee's Animal Care and Use Committee.

Histochemical and Immunohistochemical Analyses. Samples of foie gras and mouse organs obtained at necropsy were placed in 10% buffered formalin (Fisher Scientific International, Inc., Hampton, NH) and embedded in paraffin. To detect amyloid, 6-μm-thick deparaffinized sections were stained with a freshly prepared solution of alkaline Congo red and examined under polarizing microscopy. A qualitative assessment of amyloid deposition was made by an experienced microscopist (T.R.) based on the relative extent of green birefringence seen in at least 10 fields at ×20 magnification; a value of 1+, 2+, 3+, or 4+ was assigned if such material occupied, respectively, trace, minimal, moderate, or extensive portions of the sections studied, and these values were corroborated by quantitative image analyses. Electron microscopy on Epon-embedded sections stained

Table 1. Summary of the amyloidogenic potential of foie gras preparations

Group	Mice (*n*)	Treatment	Route	Positive (%)	Mean score*
1	H-2/hIL-6 (8)	Extract 1	i.v.	3 (37.5)	3+
2	H-2/hIL-6 (6)	Extract 2	i.v.	2 (33.3)	4+
3	H-2/hIL-6 (7)	Extract 3	i.v.	6 (85.7)	1.9+
4	H-2/hIL-6 (5)	PBS	i.v.	0 (0)	0
5	H-2/hIL-6 (7)	Extract 4	i.v.	7 (100)	3.1+
6	MT-1/hIL-6 (8)	Extract 4	Gavage	5 (62.5)	4+
7	MT-1/hIL-6 (7)	Extract 5	i.v.	5 (71.4)	4+
8	H-2/hIL-6 (9)	Extract 5/cooked	i.v.	5 (55.6)	1.75+
9	H-2/hIL-6 (6)	Extract 5/guanidine HCl	i.v.	2 (33.3)	0.5+
10	BALB/c (10)	Extract 5	i.v.	8 (80)	1.6+
11	BALB/c (5)	PBS	i.v.	0 (0)	0

*Mean score of amyloid-positive spleens in each group.

negatively with 1% uranyl acetate on Formvar-coated copper grids was performed with a Hitachi H-800 electron microscope (Hitachi High Technology, Pleasanton, CA). Specimens were immunostained using the avidin-biotin complex technique (Vector Laboratories, Burlingame, CA). An anti-marmoset AA murine mAb (24) and a biotinylated sheep anti-mouse Ig antiserum (BioGenex, San Ramon, CA) were used as the primary and secondary reagents, respectively, and the reactions were visualized with the Super Sensitive Link-labeled HRP Detection System (BioGenex), under conditions specified by the manufacturer.

Amyloid Extraction and Characterization. Thirty- to 80-gram portions of foie gras were cut into 0.5-cm^3 pieces and, after defatting by a series of four acetone washes, were homogenized in 0.15 M NaCl using an Omni-Mixer blender (Omni International, Marietta, GA) and centrifuged (15,000 $\times$ g) for 30 min at 4°C. This step was repeated until the OD_{280} of the supernatant was <0.10. The saline-extracted sediment was similarly homogenized in cold distilled water, and the resultant pellet was lyophilized. After reextraction with chloroform and ether, the protein was dissolved in 0.25 M Tris·HCl buffer, pH 8.0, containing 6 M guanidine HCl, reduced and alkylated, and purified by reverse-phase HPLC (25). The peak UV-absorbing fractions were dried in a vacuum centrifuge, reconstituted in sample loading buffer, and, after electrophoresis on 10% NuPage SDS/PAGE gels (Invitrogen, Carlsbad, CA), transferred to a PVDF membrane. Coomassie blue-stained bands were excised and subjected to automated sequence analysis by Edman degradation using an ABI model 494 pulsed liquid sequencer (Applied BioSystems, Foster City, CA). The amino acid sequences of tryptic peptides generated from HPLC-purified fibrillar tissue extracts were determined by MS/MS using an ion-trap instrument (Thermo Finnigan, Waltham, MA), as described previously (25).

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別紙 3-6

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 8 月 10 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			研究報告の公表状況	Quantifying the risk from ovine BSE and the impact of control strategies. Fryer, H.R. et al., Proc. R. Soc. B, 274, 1497-1503 (2007).	公表国 米国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本文献では，英国人消費者の牛海綿状脳症（BSE）感染のヒツジ肉への曝露の可能性を定量化した。感染ヒツジ内で上昇する感染力，ヒツジ間における一群内の平行伝播，及びヒツジが群れから離れてヒトの食物連鎖内へと入る速度を追跡する数学モデルを用いて，1 頭の感染ヒツジが感染ウシ 1 頭に比べ 10～1000 倍の感染物質をもたらすと予測した。また，著者らは英国内の BSE 感染が進行しているヒツジの群の数はわずか 4 群と推定した。消費者を保護するための戦略として，ヒツジ脳内で異常プリオン蛋白を検出すること，特定のリスク物質（SRM）として知られる潜在的に感染力の高い組織（腸，肝臓，胃，膵臓及びリンパ節）を屠体から取り除く法律を強化すること，6～12 ヵ月齢のヒツジが食用に使われるのを防ぐため月齢制限を設けること，遺伝子解析の実施（遺伝子型は BSE 感受性ならびに臨床疾患を発現するまでの潜伏期間の長さに影響を及ぼす）が考えられた。遺伝子解析は単独の戦略としては最も効果的であると推定されており，遺伝子解析，月齢制限，及び SRM の法律強化を組み合わせることによってリスクを最大限低減できた。	BYL-2007-0295

報告企業の意見	今後の対応
本文献の主な問題は，プリオン病についての不明点が非常に多くあるため，多くの仮定や不確実性に依存していることで，これにより将来誤った結果を導き出す可能性がある。したがって，この結果については慎重に考慮しなければならない。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報の収集に努める。

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Quantifying the risk from ovine BSE and the impact of control strategies

Helen R. Fryer[1,*], Matthew Baylis[2,†], Kumar Sivam[3] and Angela R. McLean[1]

[1]*Institute for Emergent Infections of Humans, James Martin 21st Century School, Department of Zoology, Oxford University, South Parks Road, Oxford OX1 3PS, UK*
[2]*Institute for Animal Health, Compton Laboratory, Compton, Newbury, Berkshire RG20 7NN, UK*
[3]*Veterinary Laboratories Agency, Woodham Lane, Addlestone, Surrey KT15 3NB, UK*

Although no naturally infected sheep with bovine spongiform encephalopathy (BSE) has ever been discovered, it remains possible that BSE once infected the UK sheep population, has been transmitted between sheep, and is still present today. We constructed a mathematical model to assess the current maximum theoretical exposure to consumers from BSE-infected ovine material and to estimate the risk reduction that could be achieved by abattoir-based control options if BSE-infected sheep were ever found in the national flock. We predict that, if present, the exposure to consumers from a single BSE-infected sheep would be high: one sheep, close to the end of its incubation period, is likely to contribute 10–1000 times more infectious material than a fully infectious cow. Furthermore, 30% of this exposure comes from infectivity residing in lymphatic and peripheral tissue that cannot be completely removed from a carcass.

We are 95% confident that throughout Great Britain, no more than four sheep flocks currently harbour an ongoing BSE epidemic. However, since the exposure from a single infected sheep is high, the annual human exposure from four 'typical' BSE-infected flocks could be considerable. Small reductions in exposure could be achieved by strategies based on tissue testing, a 12-month age restriction or expanded definitions of high-risk tissues. A six-month age restriction is likely to be more effective and genotype-based strategies the most effective.

Keywords: ovine; bovine spongiform encephalopathy; mathematical model; epidemiology; risk analysis

1. INTRODUCTION

Since a link was made between bovine spongiform encephalopathy (BSE) and variant Creutzfeldt–Jakob disease (vCJD; Collinge *et al.* 1996; Bruce *et al.* 1997; Hill *et al.* 1997; Scott *et al.* 1999), there has been concern that BSE may have infected sheep which were fed the same contaminated feed supplements that caused the BSE epidemic in cattle. Speculation has been driven by experimental results showing that sheep are susceptible to BSE infection from intravenous, intracerebral (ic) and oral challenge (Foster *et al.* 2001*a*,*b*; Jeffrey *et al.* 2001; Houston *et al.* 2003). Furthermore, a natural case of BSE infection in a French goat has recently been confirmed (Froissart 2004), and preliminary results suggest that a second infected goat has been located in Scotland (DEFRA 2005). However, the Veterinary Laboratories Agency (VLA) has tested 2368 ovine TSE cases from 450 flocks reporting scrapie between 2001 and 2004, and none has shown a pattern clearly indicating a case of BSE (Stack *et al.* 2006). This is promising, but it must be noted that a recent study estimated that only 38% of farmers notice scrapie actually report it (Sivam *et al.* 2003, 2006).

Even if BSE did infect the sheep population, it is questionable whether further transmission from sheep to sheep would have occurred. Neither goat case provides any insight into the likelihood of ongoing transmission because both animals were alive before comprehensive feed bans were in place in their respective countries. However, it has recently been shown that natural transmission of BSE can occur between an experimentally infected mother and her lamb (Bellworthy *et al.* 2005*a*). Scrapie does transmit between sheep and the similarities in pathogenesis of these two diseases imply that horizontal transmission of ovine BSE is a real possibility. If further transmission has occurred naturally, it is most probable that the epidemic quickly died out or is still on the decline, but there is a remote possibility that we are at the start of an ovine BSE epidemic that will continue to grow (Ferguson *et al.* 2002; Kao *et al.* 2002).

Ovine susceptibility to TSEs is largely controlled by polymorphism at the gene encoding the prion protein (PrP; Clouscard *et al.* 1995; Hunter *et al.* 1996). The principal mutations associated with susceptibility to disease are located at codons 136 (alanine (A) or valine (V)), 154 (arginine (R) or histidine (H)) and 171 (glutamine (Q), R or H). The genotype most susceptible to BSE (ARQ/ARQ) is common in British flocks.

Why should we care about an outbreak of ovine BSE? One of the main concerns centres on the distribution of infectivity through sheep tissues. While infectivity in cattle is largely confined to the nervous system (Wells *et al.* 1998), the same is not true for sheep. BSE infectivity and disease-associated prion protein have been found to be widespread throughout the body of an infected sheep

* Corresponding author (helen.fryer@zoo.ox.ac.uk).
† Present address: Veterinary Clinical Science, University of Liverpool, Leahurst, Neston, Wirral, Cheshire CH64 7TE, UK.

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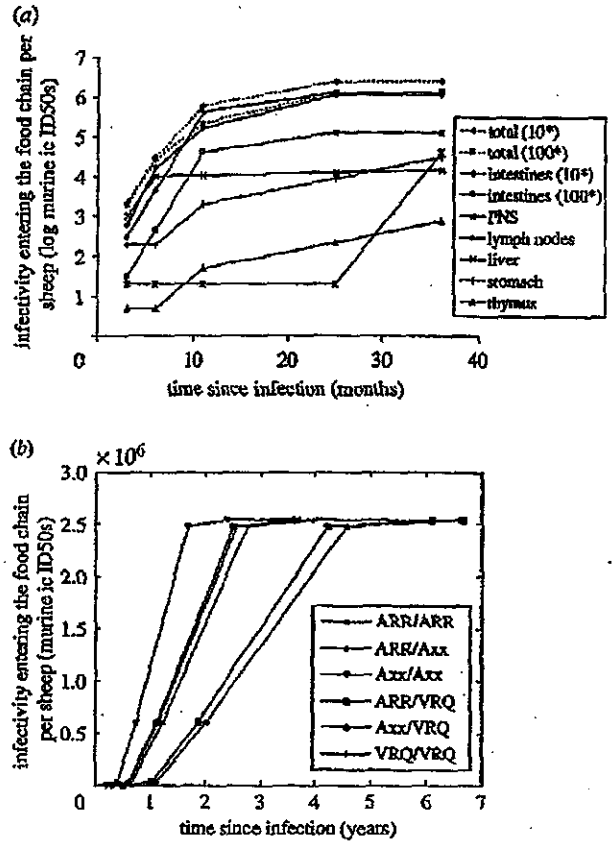

Figure 1. (*a*) Estimated infectious dose entering the food chain from one sheep over the course of an infection. (*b*) Inferred infectiousness of sheep by genotype. Infectivity entering the food chain per sheep is estimated by considering tissue weight, the proportion of each tissue that enters the food chain and infectivity in each tissue. Tissue infectivity is estimated from results of mouse bioassay experiments and tests for disease-specific PrP in sheep with natural scrapie and experimental BSE (Hadlow *et al.* 1982; Andreoletti *et al.* 2000; van Keulen *et al.* 2000, 2002; Foster *et al.* 2001*a*; Bellworthy *et al.* 2005*b*). Asterisk, the processing of the manure-stripped duodenum and jejunum into sausage casings is assumed to result in a reduction in infectivity in these tissues before consumption. The magnitude of that reduction is controversial. Two scenarios are considered: a further reduction in infectivity by a factor of 10 and a further reduction by a factor of 100. In figure (*b*), the conservative factor 10 reduction is used.

(Foster *et al.* 2001*b*; Jeffrey *et al.* 2001; Ferguson *et al.* 2002; Bellworthy *et al.* 2005*b*; Gonzalez *et al.* 2005), even in the early stages of infection. Furthermore, a number of tissues that have been shown to carry infectivity are currently eaten by humans and cannot be completely removed from a sheep carcass.

The aim of this study is to quantify the exposure to consumers to BSE-infected sheep meat. We estimate the maximum number of flocks in Great Britain that could currently be harbouring BSE and the total infectious burden that these flocks could contribute to the human food chain. We also quantify the probable impact of a range of control options. This study collates data from a wide range of sources, including the 2002 anonymous scrapie postal survey (Sivam *et al.* 2003, 2006) and the Institute for Animal Health (IAH) farm-based scrapie flock survey (Baylis *et al.* 2000), and published data on studies of natural scrapie (Hadlow *et al.* 1982; Andreoletti *et al.* 2000;

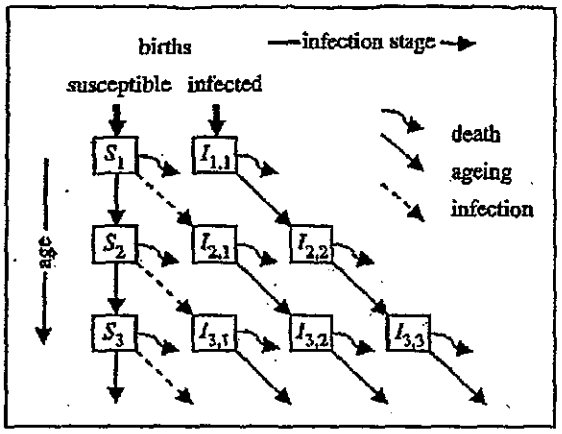

Figure 2. A within-flock model of TSE infection and disease progression. S_i represents the number of susceptible sheep in the flock aged i. $I_{i,j}$ represents the number of sheep in the flock that are aged i and have been infected for time j. These two state variables are further subdivided by sex and genotype as shown in table 1.

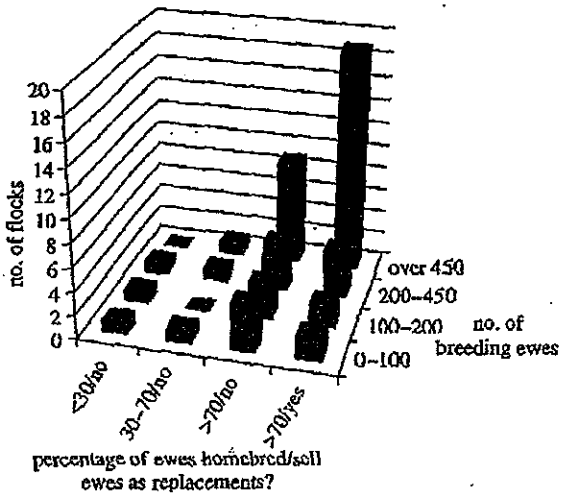

Figure 3. Size and breeding status of flocks with a scrapie epidemic. Data from the 2002 anonymous scrapie postal survey, conducted by the VLA.

van Keulen *et al.* 2000, 2002) and experimental BSE (Foster *et al.* 2001*b*; Jeffrey *et al.* 2001; Ferguson *et al.* 2002; Houston *et al.* 2003; Bellworthy *et al.* 2005*b*; Gonzalez *et al.* 2005). These data are used to parametrize a mathematical model that tracks rising infectivity within infected sheep, sheep-to-sheep transmission within a flock and the rate at which sheep leave flocks to enter the food chain.

2. MATERIAL AND METHODS

The process by which BSE-infected sheep meat could enter the food chain can be considered at three levels: the accumulation of BSE infectivity within a single sheep (figure 1); the spread of BSE infection among sheep in a single flock (figure 2); and the number of BSE-infected flocks in Britain (figure 3). We describe a mathematical model that gives a dynamic description of the infectious burden in sheep in hypothetical infected flocks. This model generates predictions about the flow of this infectious burden from infected flocks into the human food chain and allows us to compare the impact of different control options.

Table 1. Sheep genotypes: prevalence, susceptibility and incubation period.

genotype	prevalence of genotype[a] (%)		susceptibility to intracerebral BSE challenge		
	national scrapie plan screening	abattoir screening	number positive/ number challenged[b]	relative	mean incubation period after BSE intracerebral challenge[b] (years)
ARR/ARR	26.3	19.5	3/19	0.1	3.0
ARR/Axx	40.8	41.9	3/24	0.14	5.6
Axx/Axx	25.5	26.5	19/21	1	1.4
ARR/VRQ	3.4	5.5	1/11	0.14	5.1
Axx/VRQ	3.7	6.2	3/11	0.30	2.6
VRQ/VRQ	0.2	0.4	4/10	0.44	2.7

[a] Tongue *et al.* (2006).
[b] Jeffrey *et al.* (2001), Houston *et al.* (2003) and Bellworthy *et al.* (2005*b*). The allele labelled Axx represents alleles ARQ, AHQ and ARH.

(a) *BSE infectivity in a single sheep over the course of an infection*

To estimate the infectivity in a single sheep over the course of a BSE infection, we collated published data on the distribution of infectivity and abnormal PrP in natural scrapie cases and experimental BSE cases. Genotype is a strong determinant of the progression of clinical disease within an animal, but no genotype-specific tissue data are available; so, in the models used here, infectivity accumulates as a function of the average incubation period. These estimates of tissue infectivity were combined with estimates of the tissue weight and the proportion of each tissue entering the human food chain to calculate the total exposure to humans from a single infected sheep.

(b) *Within-flock spread of infectivity*

We have created a difference equation model that simultaneously encapsulates both within-sheep and within-flock dynamics of sheep TSEs. The model has full age structure and a full description of the inheritance and impact of the PrP gene.

We have developed a new classification for TSE alleles that combines ARQ, ARH and AHQ into one group of alleles which we call Axx. This simplification of PrP genetics is similar to the NSP classification, except that it has a separate group for genotype VRQ/VRQ. It allows us to model the genetics of TSE susceptibility as a single-locus, three-allele system, where the three alleles are ARR, Axx and VRQ. Properties of the six resulting genotypes are described in table 1 and figure 1.

The model has three independent variables: time, t; age, i; and time since infection, j. There are two state variables: $X_{g,i,s}(t)$, the number of susceptible sheep of genotype g, age i and sex s at time t; and $Y_{g,i,j,s}(t)$, the number of infected sheep of genotype g, age i, infected for time j and sex s at time t. The model consists of a description of disease transmission and progression that is superimposed on a model of Mendelian inheritance of the PrP gene and the demography of a flock. Data on the age (McLean *et al.* 1999) and genotype distribution of sheep in surveyed flocks were used to parametrize the genetics and demography. Sheep leave the flock to be slaughtered at an age and time-specific rate that was obtained from published slaughter statistics (DEFRA 2002). The auxiliary variable $Y^{\text{food}}_{g,i,j,s}(t)$ describes infected sheep that enter the human food chain of genotype g, age i, infected for time j and sex s at time t. Variables, parameters and equations describing the model in more detail are presented in §§ 4 and 5 of the electronic supplementary material.

The model allows for both horizontal and vertical (including perinatal) transmission of BSE. Data on attack rates after experimental challenge of sheep with BSE were used to set the relative susceptibility of different genotypes (table 1) and the absolute transmission rate was set to give a within-flock epidemic that would yield 10 clinical cases of BSE in a flock of 1067 sheep in the ninth year of an epidemic. This size of epidemic is consistent with observations of scrapie-affected flocks (see figure 4 of electronic supplementary material).

Published results on the incubation period after intracerebral BSE challenge were used to infer an incubation period for each genotype (Jeffrey *et al.* 2001; Houston *et al.* 2003; Bellworthy *et al.* 2005*b*). By analogy with scrapie (see table 4 of electronic supplementary material), we assume that natural BSE incubation periods are equal to the mean incubation period after intracerebral challenge plus 1 year (table 1). The distributions of incubation periods for each genotype follow a gamma distribution with shape parameter 7.3, which is equal to that calculated for the distribution of the age of reported scrapie cases (data from the VLA). This is consistent with the model's assumption that most sheep get infected in the first few months of life. The rate of accumulation of infectivity within an infected sheep is inferred from the infectivity data in figure 1*a* and the incubation periods in table 1.

The model's equations and parameters and detailed descriptions of parameter estimates are given in the electronic supplementary material. The model generates a dynamic description of the total burden of infectivity generated from one hypothetical flock into the human food chain as a BSE epidemic progresses.

(c) *The number of BSE flocks*

The clinical signs of scrapie and ovine BSE are indistinguishable, and it has been suggested that BSE has never been discovered in sheep because the endemic scrapie problem is masking BSE. The VLA tests reported sheep TSE cases for BSE. Samples from 2368 cases from 450 flocks (dating from 1 January 1998 to 31 May 2004) have been tested and none has shown a pattern clearly indicating BSE. This translates to a best estimate that no flocks are infected with BSE, but also provides an upper 95% confidence limit for the proportion of flocks with TSE cases that could be BSE as 0.66% (Stack *et al.* 2006).

It is important to distinguish flocks with an ongoing TSE epidemic (either BSE or scrapie) from those that have bought a TSE-infected animal that did not go on to ignite a within-flock epidemic. To estimate the number of flocks that could be harbouring an ongoing BSE epidemic, we analysed data from the 2002 scrapie postal survey. Such a flock is defined here as a holding that has had at least one case of homebred scrapie over the last year or at least two cases of scrapie in the last 5 years, of which at least one was homebred. We estimate

that, at present, there are approximately 580 flocks with an ongoing TSE epidemic (figure 3). In addition, there are another 1000 flocks that have seen scrapie over the last 5 years, but only in bought-in animals.

By combining these two calculations, we conclude that the upper 95% confidence interval (CI) for the maximum number of flocks harbouring a BSE epidemic is four. In what follows, we explore the impact of various risk reduction strategies if they were applied to four different BSE-infected flock models. The size and underlying demography of each of the four flocks are chosen to mimic four typical scrapie-affected flocks and are described in table 2 of the electronic supplementary material.

3. RESULTS

(a) *The exposure from a single sheep*

Collated data from experimental BSE infections and natural scrapie infections imply that the infectious burden in sheep infected with either TSE rises rapidly after infection and is high in peripheral tissues (see figure 1*a* and the electronic supplementary material, §3.3). Data from experimental BSE infections are largely qualitative and for quantitative measures, Hadlow's data on natural scrapie infections (Hadlow *et al.* 1982) remain the best available source of information. Although extrapolation from scrapie to BSE introduces inaccuracies, it is clear that BSE-infected sheep carry a large burden of infectivity in a wide array of tissues. Many of the tissues that carry infectivity currently enter the human food chain (see table 10 of electronic supplementary material) and some of the most heavily infected tissues are located within the muscle tissue that could not conceivably be wholly removed from a carcass. Recent studies indicate that there is also pathogenic prion protein (PrP^{Sc}) in blood (Houston *et al.* 2000; Hunter *et al.* 2002; Siso *et al.* 2006) and muscle tissue (Andreoletti *et al.* 2004).

Current legislation prevents certain ovine specified risk material (SRM) from entering the food chain. The tissues currently banned are the brain, eyes, spinal cord and tonsils of sheep over 12 months, and the spleen and ileum of all sheep. Some of the remaining tissues that carry infectivity are not used in human food. However, a number of tissues that would carry infectivity do enter the human food chain—the lymph nodes, liver, pancreas, peripheral nervous system (PNS), the duodenum and jejunum of the small intestine, and the rumen and reticulum of the stomach. It is the potential for such tissues to carry ovine BSE into the human food chain that we estimate here. This report does not consider the exposure from blood or muscle tissue as the infectivity of such tissues has never been quantified.

We have estimated that one infected sheep, two-thirds of its way through the incubation period, would contribute around 2.3 million murine ic ID50s to the food chain under current SRM legislation. The majority of this can be attributed to the exposure from lymph nodes (1.1 million) and intestines (1.2 million). At six months post-infection, the exposure from a single sheep of genotype Axx/Axx is 0.23 million murine ic ID50s. At this stage, the intestines (129 000 ic ID50s), lymph nodes (62 000 ic ID50s) and the liver (11 000 ic ID50s) are the most infectious.

How does this compare to the human exposure from cattle? One report (Comer & Huntly 2004) states that, at present, the exposure from one fully infectious cow would be approximately 27 bovine oral ID50s. Titration experiments, challenging mice (intracerebrally) and cattle (orally) with CNS tissue from naturally infected cattle provide some guide to the conversion rate between bovine oral ID50s and murine ic ID50s. Murine ic titres have been shown to vary between 10^3 and 10^5 ID50s g^{-1} CNS tissue and bovine oral titres are estimated to be 10 ID50s g^{-1} CNS tissue (SSC 2000). These figures suggest that to convert from bovine oral ID50s to murine ic ID50s requires an increase by 2–4 orders of magnitude. Even if we assume the most conservative estimate, an increase by four orders of magnitude, the infectivity from one cow corresponds to 0.27 million murine ic ID50s. This is 10 times less than the exposure from the sheep. To further put these figures into perspective, the total exposure from cattle in 2006 is estimated at around 50 bovine oral ID50s (Ferguson & Donnelly 2003; 0.005–0.5 million murine ic ID50s). Comparisons of this sort must be viewed in light of the uncertainties that remain in estimating not only the conversion rate between bovine and murine titres, but also the absolute exposure from cattle.

(b) *Exposure from four BSE-infected flocks*

Based upon results from testing reported scrapie cases for BSE, and from data provided in the scrapie postal survey, we report that the upper 95% CI estimate for the number of flocks in Great Britain with a BSE epidemic is four. Using our model, four 'typical' TSE-infected flocks would contribute around 20–30 million murine ic ID50s to the food chain each year (table 2). The vast majority of this comes from the single largest flock (see table 2 of electronic supplementary material). The estimated total exposure from cattle since 1980, of 54 million bovine oral ID50s (Comer & Huntly 2004; 5400–540 000 million murine ic ID50s), indicates that the yearly exposure from BSE-infected sheep flocks is 0.005–0.5% of the total exposure from cattle since 1980 if it is present at all.

(c) *Impact of risk reduction strategies on four infected flocks*

If BSE were discovered in the national sheep flock, what more could be done to protect consumers? In what follows, we consider the impact of various strategies that could be implemented at the abattoir level: PrP^{Sc} tests, age restrictions, tighter tissue-based controls and genotype-based exclusion (table 2). Of these options, PrP^{Sc} testing, tightened SRM legislation and a 12-month age restriction perform the worst. A six-month age restriction has intermediate impact, and genotyping strategies that involve exclusion of all but the most resistant genotypes (those carrying an ARR allele) perform the best. Even under these controls, the exposure from four sheep flocks is likely to be equivalent to the current exposure from the UK cattle population.

We have also estimated the risk reduction achieved under combinations of these strategies, the most effective of which would be a tight genotype and age-based restriction combined with stricter SRM legislation.

(i) *PrP^{Sc} testing (strategies 1 and 2)*

Strategies 1 and 2 involve testing sheep brains for PrP^{Sc}. Inevitably, the impact of such a strategy is directly linked to the sensitivity of the test used. Published data on testing infected sheep brains for PrP^{Sc} indicate that such tests

Table 2. The effect of risk reduction strategies on the infectious dose entering the food chain from four example flocks harbouring a BSE epidemic.

		infectious dose entering food chain per year (millions of murine ic ID50s)	
risk reduction strategy		ARR/ARR resistant	ARR/ARR susceptible[a]
	current legislation	23.9	27.1
1	test all for PrP^{Sc}	9.01	9.83
2	test all over 12 months for PrP^{Sc}	10.1	11.0
3	current SRM extended to all ages plus intestines, stomach, livet, thymus and 30% lymph nodes	6.66	7.58
4	only eat lamb under 12 months	9.07	9.84
5	only eat lamb under 6 months	1.64	1.76
6	eat ARR/VRQ and ARR/Axx under 12 months and all ARR/ARR	0.05	1.80
7	(6) plus eat 1% of all other sheep under 12 months	0.14	1.90
8	(6) plus eat 5% of all other sheep under 12 months	0.50	2.28
9	eat ARR/ARR under 18 months, and ARR/Axx and ARR/VRQ under 6 months	0.01	0.10
10	(3) plus only eat lamb under 12 months	1.99	2.17
11	(3) plus only eat lamb under 6 months	0.40	0.43
12	(6) plus (3)	0.01	0.57
13	(9) plus (3)	0.003	0.03

[a] ARR/ARR sheep have a relative susceptibility of 0.1 and mean incubation period of 4 years.

perform poorly in the early stages of infection; the earliest that PrP^{Sc} has been detected in a TSE-infected brain is at 9–10 months post-infection (Andreoletti *et al.* 2000; van Keulen *et al.* 2002), while in the same experiments, PrP^{Sc} was detected in lymph nodes at only 2–3 months post-infection. Based on published data (Jeffrey *et al.* 2001; van Keulen *et al.* 2002; Bellworthy *et al.* 2005*b*), we make conservative estimates of test sensitivity (see table 7 of electronic supplementary material) that are reflected in poor performance from these strategies, reducing the infectious load entering the food chain from four flocks by around 60%.

(ii) *Tighter SRM (strategy 3)*

We have considered the impact of tightening the SRM legislation to span a wider range of tissues that pose a potential risk to consumers at present. In practice, not all infectious tissues could be removed from a carcass. The PNS and the lymphoreticular system extend throughout the carcass and full removal would be costly, if not impossible.

Here, we model the risk reduction that is achieved by adding the liver, pancreas, stomach, intestines and 30% of the lymph nodes to the list of SRM. Under this assumption, the exposure from a single sheep, two-thirds of its way through an infection, would drop from 2.3 to 0.8 million murine ic ID50s. Most of this reduction is attributable to the further removal of the intestines and lymph nodes. The exposure from a six-month infected Axx/Axx sheep would drop from 0.20 to 0.04 million murine ic ID50s. At this age, removal of the liver is also significant.

The exposure from four flocks, under this tightened SRM strategy, is reduced by 72%. The residual 28% results from the difficulty in removing the PNS and all the lymph nodes, both of which have a high level of infectivity, particularly later on in the incubation period.

(iii) *Age restrictions (strategies 4 and 5)*

We modelled the impact of age restrictions that prevent sheep over 12 or 6 months from entering the human food chain. These strategies would reduce the infectious load from four flocks by 63 and 93%, respectively. These strategies perform poorly because Axx homozygous sheep (27% of the UK flock) are easily infected and have a short incubation period. They are therefore likely to bear a heavy burden of infectivity by six months of age. At 12 months of age, these sheep are likely to be nearly halfway through their incubation period and harbouring 1.1 million murine ic ID50s.

(iv) *Genotyping combined with age restrictions*

Sheep genotype confers susceptibility to TSE infection and affects the time from infection to onset of clinical disease. Sheep of different genotypes thus pose different levels of risk to consumers. Possible control options could restrict the genotypes that may enter the food chain.

Eat only ARR/ARR (all ages) and ARR/— under 12 months (strategies 6–8). The ARR allele has long been associated with resistance to ovine TSEs. No ARR homozygous sheep has yet been discovered with clinical scrapie, though a number of atypical scrapie infections have been found in sheep with this genotype (Everest *et al.* 2006), and experiments suggest they are also the least susceptible to BSE. We have modelled the impact of a control option that involves allowing only ARR homozygous sheep (all ages) and ARR heterozygous sheep that are under 12 months old into the food chain. In the event that ARR homozygotes are completely resistant, there is a 99.8% risk reduction, making it one of the more effective strategies considered here. It is, however, still not perfect because ARR heterozygotes are susceptible and could pose a risk, even before 12 months of age.

It is debatable whether or not ARR homozygous sheep would, in fact, be resistant to BSE under natural circumstances as oral and intracerebral challenges have proved successful (Houston *et al.* 2003; Gonzalez *et al.* 2005; Andreoletti *et al.* 2006). Clearly, this control option is less effective if ARR homozygotes are susceptible to natural infection. This is reflected in a less promising

estimate that the exposure is reduced by only 93% under the assumption that these sheep are marginally more resistant than ARR/Axx sheep and have an average incubation period of 4 years.

If genotype testing were imperfect (so that a percentage of sheep of other genotypes were mistakenly allowed into the human food chain), then these strategies become significantly less effective (strategies 7 and 8).

Eat ARR/ARR under 18 months and ARR/— under six months (strategy 9). A tighter genotyping strategy could also include an age restriction of, say, 18 months on ARR homozygotes and six months on ARR heterozygotes. This would be more effective, reducing the exposure by 99.9 and 99.6%, to 0.01 and 0.10 million murine ic ID50s, if homozygous sheep are resistant and susceptible, respectively. These figures lie within the estimated range for the total exposure from cattle in 2006, 0.005–0.5 million murine ic ID50s.

(v) *Combination strategies (strategies 10–13)*

Not surprisingly, combination strategies are more effective than single strategies. We considered four strategies that combined maximum SRM removal with age and genotype restrictions. The tightest of these (strategy 13) allows only ARR homozygotes under 18 months and ARR heterozygotes under six months into the food chain and further imposes a maximum realistic SRM removal scheme. If ARR homozygotes are completely resistant to BSE infection, then this strategy reduces infectivity in the human food chain by four orders of magnitude. The strategy has 10-fold less impact if ARR homozygotes are somewhat susceptible.

4. DISCUSSION

We estimate that there are at most only four flocks currently harbouring a BSE epidemic in Britain, but that even a single BSE-infected sheep could pose a considerable risk to consumers, contributing 10–1000 times as much infectivity in the human food chain as a fully infectious cow. Furthermore, 30% of the exposure from sheep comes from infectivity residing in lymph nodes and the PNS—tissues that cannot feasibly be entirely removed from a carcass.

The exposure from four 'typical' BSE-infected sheep flocks each year could be considerable. Our models predict that only a small reduction in exposure could be achieved by a PrP^{Sc}-testing based strategy, a 12-month age restriction or a tightened tissue-based strategy. A six-month age restriction is likely to be more effective and genotype-based strategies, which allow only the most resistant genotypes to enter the food chain, will achieve the greatest reduction in risk to consumers.

All the options discussed here are currently under consideration by the authorities in contingency planning for the event that ovine BSE is discovered. Such decisions, however, must also take into account the predicted cost and feasibility of different plans. Genotyping all sheep in the UK would be extremely expensive, if at all possible or accurate, on such a large scale. Furthermore, it would remove a high proportion of sheep from the food chain, which would also be the disadvantage of having a strict age-based cut-off. Testing sheep for PrP^{Sc} would be less expensive overall as it would not waste vast numbers of uninfected sheep. Tighter SRM-based strategies are also likely to be relatively cheap since only an extension of the existing SRM procedure would be required.

Our calculations rest upon a straightforward mathematical model, but are necessarily data hungry in a situation where not all the relevant data have yet been gathered. The modelling exercise usefully highlights the most glaring gaps in our knowledge. For calculating the infectious load produced by a BSE-infected sheep flock, the most important new data would be quantified BSE infectivity in different tissues in sheep of different genotypes at 6 and 12 months of age (after infection very close to birth). For comparisons of the impact of different risk reduction strategies, information on the sensitivity of proposed tests by genotype, tissue and time since infection would be particularly useful. The comparative risk of ovine and bovine BSE requires further analysis of the conversion rate from bovine oral ID50s to murine ic ID50s. The range used here of 2–4 orders of magnitude probably does not reflect all of the uncertainty surrounding this estimate. Furthermore, comparisons between the exposure from bovine and ovine BSE must be viewed in the light of the uncertainties surrounding estimates of the exposure from cattle.

Although gaps exist in our detailed knowledge of the dynamics of BSE infectious load in infected sheep, our conclusions are robust to the uncertainties that remain and provide best estimates of the exposure from ovine BSE and the effectiveness of control options which can be used in contingency planning. This is the only study that assesses the impact of genotype-based strategies and compares them with other options.

Our main conclusion is that we should remain vigilant of ovine BSE, simply because even a single recently infected sheep is likely to harbour considerable infectivity throughout the carcass, including in tissues that could not feasibly be removed at the abattoir. Furthermore, despite much positive news in recent years, a slowly developing ovine BSE epidemic is not inconceivable and the genotype of sheep that would most easily be infected and in which disease progresses most quickly is very common in our sheep flocks.

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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

○海綿状脳症諮問委員会(SEAC)、vCJDと歯科治療に関する意見書
英国保健省はSEACに歯科治療処置を介したvCJD伝播のリスク算出を目的とした初期研究の成果についての助言を求めた。
歯組織にプリオン伝播性、感受性がある場合、歯科治療器具によるvCJDの二次伝播が起こりうる。輸血や脳外科手術と異なり、歯科治療は実施数が多く、通常の健康状態の人も受けている。また、記録も不完全なため伝播が起こった場合の追跡と感染防御が困難である。
現時点で歯科処置によるvCJD伝播は起こっていない。歯科治療器具を介したvCJD伝播は、舌扁桃を偶然切った場合と歯髄に接触する処置を行う場合とがあり、後者の方がリスクは高いとみられる。このため、SEACは歯髄治療用器具の使い捨てを勧告した。これが順守されれば、ヒト―ヒト間の持続的感染拡大を防止することが出来るだろう。
初期研究では、歯科処置によるvCJD伝播のリスクがこれまで考えられていたよりも高いことが示唆された。この研究は高用量の感染性物質に暴露された動物モデルを使用した不完全なものであり、またヒトの歯組織の感染性を示すデータはないものの、これらの知見はvCJDが歯科処置によって効率的に伝播されうることを示している。ヒトの歯組織を用いた感染性試験が現在行われており、より正確なリスク算定が可能になるだろう。
公衆衛生上の影響についてのより綿密な考察と、さらなるリスク減少手段の特定のため、全ての歯科治療のリスクについて詳細で包括的な評価を早急に行うことも重要である。評価を迅速に行うために専門家のグループを招集するという保健省の提案は歓迎される。また、伝播のリスクを減少できる新しい除染技術について早急な評価を行うことを考慮すべきである。

報告企業の意見	今後の対応
英国海綿状脳症諮問委員会が、英国保健省の要請に応じてvCJDと歯科治療に関する意見書を発表し、歯科治療のリスク評価、新たな除染技術の評価を求めた。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

SEAC

Position Statement

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vCJD and Dentistry

Issue

1. The Department of Health (DH) asked SEAC to advise on the findings of preliminary research aimed at informing estimates of the risk of variant Creutzfeldt-Jakob Disease (vCJD) transmission via dentistry.

Background

2. Prions are more resistant than other types of infectious agent to the conventional cleaning and sterilisation practices used to decontaminate dental instruments[1]. Appreciable quantities of residual material may remain adherent to the surface after normal cleaning and sterilisation[2]. Therefore, if dental tissues are both infectious and susceptible to infection, then dental instruments are a potential mechanism for the secondary transmission of vCJD. Dentistry could be a particularly significant route of transmission for the population as a whole, due to the large number of routine procedures undertaken and also because dental patients have a normal life expectancy. This is in contrast with other transmission routes, such as blood transfusion and neurosurgery, where procedures are often carried out in response to some life-threatening condition. Additionally, the ubiquity of dental procedures and the lack of central records on dental procedures means that should such transmission occur, then it would be difficult to detect and control.

3. Cases of vCJD transmission arising from dental procedures have been reported to date [3] . Previous DH risk assessments[4,5] have focused on two possible mechanisms for the transfer of vCJD infectivity via dental instruments; accidental abrasion of the lingual tonsil and endodontic procedures that involve contact with dental pulp. In considering these assessments, SEAC agreed that the risk of transmission via accidental abrasion of the lingual tonsil appears very low. However, the risk of transmission via endodontic procedures may be higher and give rise to a self sustaining vCJD epidemic under circumstances where (i) dental pulp is infective, (ii) transmission via endodontic instruments is efficient and (iii) a large proportion of vCJD infections remain in a subclinical carrier state (SEAC 91, February 2006). In light of this, SEAC advised that restricting endodontic files and reamers to single use be considered [6]. SEAC recommended reassessment of these issues as new data emerge.

New research

4. Preliminary, unpublished results of research from the Health Protection Agency, aimed at addressing some of the uncertainties in

otection Agency, aimed at addressing some of the uncertainties in e risk assessments, were reviewed by SEAC (SEAC 97, May 2007). e prion agent used in these studies is closely related to the vCJD ent. This research, using a mouse model, shows that following oculation of mouse-adapted bovine spongiform encephalopathy SE) directly into the gut, infectivity subsequently becomes despread in tissues of the oral cavity, including dental pulp, salivary ands and gingiva, during the preclinical as well as clinical stage of sease.

It is not known how closely the level and distribution of infectivity in e oral cavity of infected mice reflects those of humans infected with JD, as there are no comparable data from oral tissues, in particular ntal pulp and gingiva, from human subclinical or clinical vCJD ses[7]. Although no abnormal prion protein was found in a study of man dental tissues, including dental pulp, salivary glands and ngiva from vCJD cases , the relationship between levels of infectivity d abnormal prion protein is unclear[8]. Infectivity studies underway ing the mouse model and oral tissues that are presently available om human vCJD cases will provide some comparable data. On the sis of what is currently known, there is no reason to suppose that e mouse is not a good model for humans in respect to the stribution of infectivity in oral tissues. Furthermore, the new data are nsistent with published results from experiments using a hamster rapie model[9] .

A second set of experiments using the same mouse model showed at non-invasive and transient contact between gingival tissue and e dental files contaminated with mouse-adapted BSE brain mogenate transmits infection very efficiently. It is not known how ficient gingival transmission would be if dental files were ntaminated with infectious oral tissues and then subsequently eaned and sterilised, a situation which would more closely model man dental practice. Further studies using the mouse model that ould be more representative of the human situation, comparing oral sues with a range of doses of infectivity, cleaned and sterilised files d the kind of tissue contact with instruments that occurs during ntistry, should be considered.

SEAC considered that the experiments appear well designed and the nclusions justified and reliable, while recognising that the research is complete and confirmatory experiments have yet to be completed. It recommended that the research be completed, submitted for peer-view and widely disseminated as soon as possible so others can nsider the implications. Nevertheless, these preliminary data crease the possibility that some oral tissues of humans infected with JD may potentially become infective during the preclinical stage of e disease. In addition, they increase the possibility that infection uld potentially be transmitted not only via accidental abrasion of the gual tonsil or endodontic procedures but a variety of routine dental ocedures. Implications for transmission risks

The new findings help refine assumptions made about the level of fectivity of dental pulp and the stage of incubation period when it comes infective in the risk assessment of vCJD transmission from [10]

the reuse of endodontic files and reamers[11]. For example, if one patient in 10 000 were to be carrying infection (equivalent to about 6 000 people across the UK – the best current estimate[11]), the data suggest that in the worst case scenario envisaged in the risk assessment, re-use of endodontic files and reamers might lead to up to 150 new infections per annum. It is not known how many of those infected would go on to develop clinical vCJD. In addition, transmission via the re-use of endodontic files and reamers could be sufficiently efficient to cause a self-sustaining vCJD epidemic arising via this route.

9. These results increase the importance of obtaining reliable estimates of vCJD infection prevalence. Data that will soon be available from the National Anonymous Tonsil Archive may help refine this assessment and provide evidence of the existence and extent of subclinical vCJD infection in tonsillectomy patients. Further data, such as from post mortem tissue or blood donations, will be required to assess prevalence in the general UK population[12].

10. Recent guidance issued by DH to dentists to ensure that endodontic files and reamers are treated as single use[13] is welcomed and should, as long as it is effectively and quickly implemented, prevent transmission and a self-sustaining epidemic arising via this route. However, the extent and monitoring of compliance with this guidance in private and National Health Service dental practice is unclear.

11. The new research also suggests that dental procedures involving contact with other oral tissues, including gingiva, may also be capable of transmitting vCJD. In the absence of a detailed risk assessment examining the potential for transmission via all dental procedures, it is not possible to come to firm conclusions about the implications of these findings for transmission of vCJD. However, given the potential for transmission by this route serious consideration should be given to assessing the options for reducing transmission risks such as improving decontamination procedures and practice or the implementation of single use instruments.

12. The size of the potential risk from interactions between the dental and other routes of secondary transmission, such as blood transfusion and hospital surgery, to increase the likelihood of a self-sustaining epidemic is unclear.

13. It is likely to be difficult to distinguish clinical vCJD cases arising from dietary exposure to BSE from secondary transmissions via dental procedures, should they arise, as a large proportion of the population is likely both to have consumed contaminated meat and undergone dentistry. However, an analysis of dental procedures by patient age may provide an indication of the age group in which infections, if they occur, would be most likely to be observed. Should the incidence of clinical vCJD cases in this age group increase significantly, this may provide an indication that secondary transmission via dentistry is occurring. Investigation of the dental work for these cases may provide supporting data. There is no clear evidence, to date, based on surveillance or investigations of clinical vCJD cases, that any vCJD cases have been caused by dental procedures but this possibility cannot be excluded.

nnot be excluded.

nclusions

. Preliminary research findings suggest that the potential risk of ansmission of vCJD via dental procedures may be greater than eviously anticipated. Although this research is incomplete, uses an imal model exposed to relatively high doses of infectivity, and there e no data from infectivity studies on human oral tissues, these dings suggest an increased possibility that vCJD may be relatively ficiently transmitted via a range of dental procedures. Ongoing fectivity studies using human oral tissues and the other studies ggested here will enable more precise assessment of the risks of JD transmission through dental procedures.

. Guidance was issued to dentists earlier this year recommending at endodontic files and reamers be treated as single use which, ovided it is adhered to, will remove any risk of a self-sustaining idemic arising from re-use of these instruments. To minimise risk it critical that appropriate management and audit is in place, both for HS and private dentistry.

. It is also critical that a detailed and comprehensive assessment of e risks of all dental procedures be conducted as a matter of urgency. hile taking into account the continuing scientific uncertainties, this ll allow a more thorough consideration of the possible public health plications of vCJD transmission via dentistry and the identification of ssible additional precautionary risk reduction measures. The sessment will require continued updating as more evidence becomes ailable on the transmissibility of vCJD by dental routes, and on the evalence of infection within the population. A DH proposal to nvene an expert group that includes dental professionals to expedite ch an assessment is welcomed. Given the potential for transmission a dentistry, consideration should be given to the urgent assessment new decontamination technologies which, if proved robust and fective, could significantly reduce transmission risks.

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医薬品
医薬部外品　研究報告　調査報告書
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別紙 3-4

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 5 月 17 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			研究報告の公表状況	vCJD and blood transfusion in the United Kingdom Hewitt, P. Transfusion clinique et biologique, 13, 312-316 (2006)	公表国 英国	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
本稿の大部分は，昨年 Vox Sanguinis で発表された同一の著者による総説〔第 8 回感染症定期報告で報告（BYL-2007-0263)〕でも報告されている。追加情報はないが，恐らく，血漿由来製剤による変異型クロイツフェルト・ヤコブ病の感染リスクは血液成分輸血によるリスクよりもはるかに低い，という一般的な概念を支持している。	BYL-2007-0285

報告企業の意見	今後の対応
これまでに，血友病患者及び英国で採取された血漿由来製剤の大量投与を受けた患者での vCJD 症例は 1 例も報告されていないことは，血液製剤による vCJD 伝播のリスクは極めて低いことを示唆している。弊社の血漿分画製剤及びコージネイト FS 又はコージネイト FS バイオセットの製造工程培地に使用されている血漿分画成分は，英国よりもリスクがかなり低い米国で採取された血漿で製造されている。その上，これら血漿分画製剤の製造工程では，大幅にプリオンを除去することが実験的に確認された病原体除去工程を導入している。	現時点で新たな安全対策上の措置を講じる必要はないと考える。

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Original article

vCJD and blood transfusion in the United Kingdom

Variant de la maladie de Creutzfeldt-Jakob et transfusion sanguine au Royaume-Uni

P. Hewitt[1]

National Blood Service, 75, Cranmer Terrace, London SW17 ORB, UK

Available online 22 December 2006

Abstract

A study was set up in the UK in 1997 to examine whether there is any evidence that variant Creutzfeldt–Jakob disease (vCJD) is transmitted by blood transfusion. To date, the study has identified three probable cases of vCJD transmission by blood transfusion, including two clinical cases and one pre- (or sub-) clinical case.
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Résumé

Une étude a débuté en 1997 au Royaume-Uni, afin de déterminer si le variant de la maladie de Creutzfeldt-Jakob pouvait être transmis par transfusion sanguine. Actuellement, cette étude a permis d'identifier trois cas probables de transmission, incluant deux cas cliniques et un cas pré- ou subclinique.
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Keywords: Blood transfusion; Transmission; vCJD

Mots clés : vMCJ ; Transfusion sanguine ; Risque

1. Introduction

Although rare, prion diseases have caught the public attention in recent years due to the emergence of new forms, such as ovine spongiform encephalopathy (BSE) and variant Creutzfeldt-Jakob disease (vCJD). BSE occurred as an epidemic in UK cattle in the 1980s and 1990s, and during that time a very large number of BSE-infected cattle carcasses would have been likely to enter the human food chain, possibly giving rise to human infection with BSE (vCJD) through contaminated meat products.

The commonest form of human prion disease is the sporadic form of Creutzfeldt-Jakob disease (sCJD), which occurs most commonly in the seventh decade of life. sCJD occurs as a worldwide disorder affecting around one patient per million of the population each year. The clinical features are those of a rapidly progressive dementia with a range of other neurological abnormalities including visual abnormalities and movement disorders (particularly ataxia and myoclonus), resulting in death around 4 months after the disease onset. Until 2004, it was generally accepted that CJD had not been transmitted by blood transfusion. Small studies, both case–control studies and lookback investigations, had not detected any link between sCJD and blood transfusion.

2. CJD surveillance

In 1990 the National CJD Surveillance Unit (NCJDSU) was established in the UK with the aim of identifying all cases of CJD. Suspected cases of CJD are referred to the NCJDSU from

E-mail address: patricia.hewitt@nbs.nhs.uk (P. Hewitt).
[1] NBS Consultant Specialist in Transfusion Microbiology.

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targeted professional groups, including neurologists and neuropathologists. A neurologist from NCJDSU reviews the case, the investigation results, and the neuropathological material when available. Cases are classified according to standard diagnostic criteria. Onset of clinical symptoms for CJD cases are estimated to the nearest month by NCJDSU after reviewing the case notes. Details of past medical history, including blood donation or transfusion, are obtained from the family of suspected cases.

As of August 2006, there have been 162 cases of vCJD in the UK: the majority have been confirmed by neuropathological examination. Six of the cases are alive. Modeling by statisticians suggests that the peak of the epidemic occurred in 1999, and confirms that cases continue to decline year-on-year since then. It is possible, however, that there could be further peaks due to cases in individuals of different genetic make-up, or to secondary spread through other routes such as blood transfusion or exposure through surgery.

The median age at onset of the vCJD cases is 26 years and the median age at death is 28 years. The illness has a median duration of 14 months, which is very different from sCJD. Although most cases are in the younger age group, and the youngest case was age 12 at diagnosis, there have been cases in those over the age of 60, and the oldest diagnosed case was age 74.

Risk factors for vCJD, determined by detailed epidemiological investigation, include a young age, being homozygous for methionine at codon 129 of the prion protein gene, and having had surgical treatment in the past. There is a higher prevalence of the disease in the northern half of the UK (Scotland and northern England) compared with the rest of the UK (Northern Ireland, Wales and southern England).

3. Surveillance of vCJD and blood transfusion

Following the identification of vCJD a collaborative study was established in 1997 between the NCJDSU and the four UK blood services (UKBS). The study (the Transfusion Medicine Epidemiology Review, TMER) was designed to investigate whether there is any association between any type of CJD (sporadic, familial and variant) and blood transfusion. The study was granted ethical approval by the local Research Ethics Committee and aims to include all cases of CJD reported in the UK. For the purpose of this paper, only vCJD will be considered.

All patients with vCJD (probable or confirmed) who are old enough to have donated blood (>17 years of age) are notified to UKBS at diagnosis, whether or not there is a known history of blood donation. This decision was made on public health grounds, because information obtained from third parties (family or friends) is less reliable than that obtained direct from the individual. Upon receipt of notification from the NCJDSU, the UKBS searches for donor records relating to that case, using name, date of birth and previous addresses as identifiers. For cases reported as blood donors, information on dates and places of donation is also used to help locate past donor records. Where donor records are found, all components issued to hospitals are identified and their fate determined, as recorded in hospital blood transfusion laboratory records. At this stage, no check is made of the medical records relating to identified recipients and it is assumed that the blood component was transfused to that recipient. Details of the recipients (name and date of birth) are checked against the NCJDSU register to establish if there is a match between these individuals and patients who have developed CJD. Checks continue to be made at three-monthly intervals, to identify any cases which develop subsequently. Recipients details are also flagged with the Office of National Statistics (ONS), which provides a copy of the death certificate to NCJDSU for checking to establish date and certified cause of death.

4. Evidence from animal studies

After the start of the study, in 2002, findings from sheep studies were published, indicating that BSE and scrapie can be transmitted by blood transfusion [1]. It would be reasonable to assume that these findings could also apply to human TSEs, and in particular vCJD, even though there is no evidence that sCJD has been transmitted by blood transfusion. The UK is the only country where a significant outbreak of vCJD has occurred and is in a unique position to study this question which has important implications for public health policy.

5. Identification of donors

As of 1st September 2006 there were a total of 162 vCJD cases on the NCJDSU register. Cases who were old enough to have been potential blood donors (150) have been notified to UKBS. In 31 cases families reported a probable history of blood donation at various times in the past, although there is variation in the details of available information and the confidence of families in donation history.

Donor records were found for 20/31 vCJD cases reported by relatives as blood donors and for four additional cases with no reported donation history. Of the 24 found, 18 vCJD cases (12% of the total eligible to donate blood) were confirmed to have donated labile blood components, with the number of components made and issued for use in UK hospitals ranging from 1 to 14 per donor. Six vCJD cases were registered as donors, but had not donated labile blood components for a variety of reasons.

The search for donor records was negative in 11/31 (35%) vCJD cases reported as putative donors, three of whom allegedly donated well before the onset of the BSE epidemic in the 1980s. In most of these cases the information provided was minimal, except in one case where relatives were confident that regular donations (up to 50) had been made in the years leading up to 1993. Despite extensive searches no records were found and no explanation has been found for the lack of records, although discrepancies in some of the details suggest that the history was not as certain as initially thought.

5.1. Labile components issued to hospitals

Over the period 1981–2004, 66 labile components originating from 18 donors were issued to UK hospitals and transfused to patients according to blood transfusion laboratory records. A further nine components issued between 1982 and 1996 could not be traced by the relevant hospital. Fifty-six recipients (85%) received red cells or whole blood, seven (11%) were transfused with labile plasma components or derivatives and three (4%) received pooled platelets made according to UK specifications in which the buffy-coat preparation containing platelets from the implicated vCJD donor was pooled with buffy coats from three other donors and resuspended in plasma from one of the four donations. Nearly half of the red cell recipients received red cells which had been leucocyte-depleted by pre-storage filtration to $<5 \times 10^6$ leucocytes per unit (in 99% of units with 95% statistical confidence according to UK guidelines) after the introduction of universal leucocyte depletion of the UK blood supply in 1999.

5.2. Recipients of blood components

Patient identifiers are available for 66 recipients who received blood from 18 different donors who went on to develop vCJD. None of the 66 recipients had themselves donated blood between receiving their transfusion and early 2004 when the UKBS implemented a policy of excluding all donors transfused in the UK since 1st January 1980 as a vCJD risk reduction measure. As would be expected in a recipient population, 41 (62%) recipients were aged over 60 years at the time of transfusion and were not eligible to donate under the donor selection guidelines in place before 2004. All living recipients (N=24) have been informed of their risk and advised not to donate blood, tissues or organs. Three instances of probable transfusion transmitted vCJD infection have occurred, including two confirmed clinical cases and one pre or subclinical infection. All three of these recipients have died. Figs. 1,2 show the survival period for dead (transfusion to death) and live recipients (transfusion to 2006) of vCJD components, respectively, according to the interval between transfusion and onset of clinical symptoms in the donor.

5.3. Dead recipients

Forty-two recipients are known to be dead: mean age at death 66±19 years. Around half (N=21) of these recipients died within a year of receiving their transfusion, with only seven surviving for more than 5 years. Two recipients, who died 4 and 11 months after transfusion, had "Dementia" recorded on the death certificate, but neither case had features to suggest vCJD. All other recipients were certified as dying of causes unrelated to vCJD, except for the two cases who are now known to represent probable transfusion transmission of vCJD. In the first case, the cause of death was recorded as "1A Dementia and II. Prostate Cancer". This person had received a non-leucodepleted red cell component from a donor later confirmed neuropathologically as suffering from vCJD [2]. The recipient received the transfusion of red cells 6 1/2 years before onset of clinical symptoms, and was a methionine homozygote at codon 129 of the human prion protein gene (*PRNP*). The donor to this individual also had a neuropathological diagnosis of vCJD, with clinical onset approximately 40 months after donating. A second red cell recipient received non-leucodepleted red cells from a different donor who developed clinical symptoms approximately 18 months after donating and was later diagnosed with neuropathologically confirmed vCJD. This recipient died 5 years after transfusion without any clinical symptoms of vCJD, and was found to have protease-resistant prion protein (PrP^{res}) in the spleen and one lymph node (but not in the brain) at post mortem [3]. The recipient was a codon 129 *PRNP* heterozygote and the case is thought to

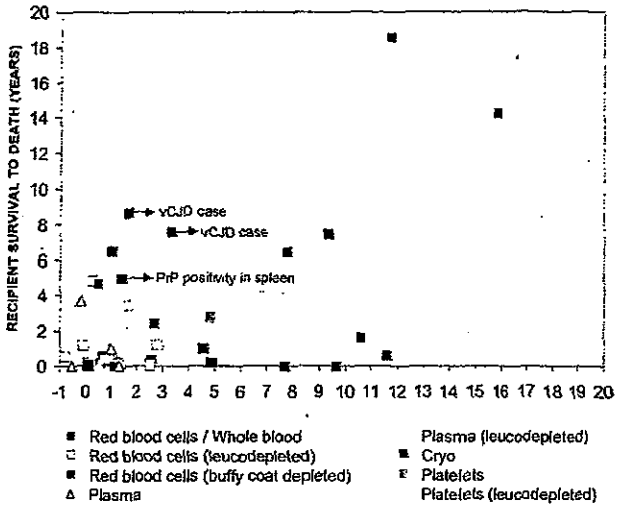

Fig. 1. Survival period (transfusion to death) for recipients of vCJD components, according to interval between blood donation and onset of clinical symptoms in the donor.

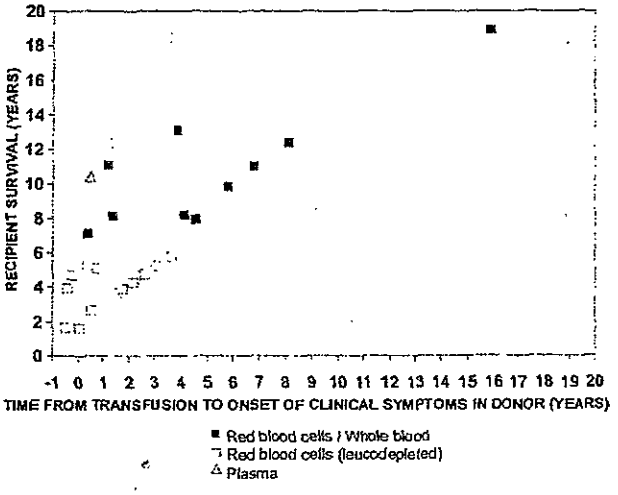

Fig. 2. Current survival period (since transfusion to June 2006) for recipients of vCJD components according to interval between blood donation and onset of clinical symptoms in the donor.

represent pre- or sub-clinical infection. The third case of probable transmission of vCJD infection by blood transfusion involved a recipient who received a transfusion of red cells 7 years and 10 months before onset of clinical symptoms [4] of vCJD. The recipient had received a large number of donor exposures for a major bleeding episode during surgery, but one of the red cell donors developed vCJD approximately 21 months after the donation, and the recipient was a codon 129 *PRNP* methonine homozygote.

5.4. Live recipients

Twenty-four recipients (%) are alive: mean age of 63 ± 19 years. Half of the live recipients were transfused with components from donors whose donations were made within 20 months of clinical onset of vCJD, in some cases around the time of development of the first signs of clinical illness. These donors would have appeared healthy and passed the normal medical checks as being fit to donate. Sixteen recipients have survived longer than 5 years, with six surviving > 10 years. These patients, mean age currently 61±19 years, were given blood from donors who developed vCJD symptoms at intervals ranging from around 5 to 191 months after making the donation.

5.5. Plasma for UK fractionation

Twenty-five units of plasma originating from 11 different donors, bled between 6 months and 18 years before onset of clinical vCJD symptoms, were supplied for UK fractionation during the period 1986–1998. Product batches manufactured from 23 plasma units derived from nine donors have been traced. The fate of batches of product derived from the two remaining plasma donations, from two different donors, is still being established. The fate of batches of products has not been traced to individual recipients as part of this study. It is known, however, that Hemophilia Centers have traced the ultimate fate of the batches of Factor VIII. It is also known that no case of vCJD has been identified in a patient with Hemophilia in the UK.

6. vCJD cases with history of transfusion

Eleven vCJD cases were reported to have received past blood transfusions between 1962 and 1999. A further case, excluded from further analysis, received a blood transfusion after onset of illness. For two cases, hospital records showed that they had not been transfused. No hospital records could be found for another two cases reported to have been transfused in 1962 and 1971, respectively. Hospital transfusion records were found for seven vCJD cases (64% of those reported as transfused) who had been transfused with components donated by 181 donors (169 identified), ranging from 2 to 103 per case. The latter case (103 donor exposures) also received a solid organ transplant and another case (the third case of probable transmission), received 59 donor exposures. To date, two donors are also registered on the NCJDSU database as vCJD cases. These are the donors of the two clinical cases of transfusion transmitted vCJD referred to previously (see vCJD cases with history of donation). No additional linkages between recipients and donors were identified through investigation of the cases in blood recipients, and no infected donor has been identified in the remaining five cases.

7. Discussion

This study has identified three instances in which a recipient of a transfusion derived from a 'vCJD' donor have developed infection with vCJD, including two clinical cases and one pre- or sub-clinical infection [2–4]. These are three different donor/recipient pairs. In view of the small size of the total at risk recipient population (N=66) and the background mortality rate for vCJD in the general UK population (0.24 per million per annum), these observations provide strong evidence that vCJD can be transmitted from person to person through blood transfusion. This finding has had important implications for public health policy nationally and internationally.

The risk of developing vCJD infection in the surviving recipient population is significant but cannot be precisely estimated because of variables including the timing of blood donation in relation to clinical onset in the donor, the influence of the codon 129 genotype of donor and recipient and the effect of the introduction of leucodepletion in 1999. Furthermore, the currently observed number of infections in the recipient population may be an underestimate as some surviving recipients may yet develop vCJD and there is limited available information on the outcome in the cohort of deceased recipients; a significant proportion of these individuals may not have survived long enough to express clinical disease even if infected. Extrapolating from the three observed infections in the total recipient population is likely to lead to an underestimate of the overall risk of transfusion transmission of vCJD, although the introduction of leucodepletion in 1999 may have reduced the risk to recipients transfused after this date.

A further important variable in estimating individual risk is the time from blood donation to clinical onset in the donor and, although evidence from animal studies in relation to this issue is conflicting, it is likely that an extended gap between blood donation and clinical onset in the donor will reduce the risk of transfusion transmission.

All tested clinical cases of vCJD have been methionine homozygotes at codon 129 of PRNP, but the individual with 'pre-clinical' transfusion transmitted infection was heterozygous at this locus [3], indicating that individuals with this genotype are susceptible to secondary infection with vCJD. Except for the three cases infected through blood transfusion, the codon 129 genotypes of the recipient population are not known. Risk may vary according to genetic background, but it cannot be assumed that some recipients will possess an absolute genetic barrier to infection.

The analysis of vCJD cases with a history of blood transfusion has identified over 100 donors to these cases, although the

great majority were linked to only two vCJD cases. A risk assessment has suggested that these donors are themselves at significant risk of developing vCJD and these individuals have been informed of this risk and have been advised not to act as blood or organ donors. To date none of these individuals have developed vCJD, with the exception of the two donors already linked to the two clinical cases of vCJD described above.

Plasma derived from vCJD cases has been used in the production of plasma derived products, including clotting factors and immunoglobulin. To date, there is no evidence that vCJD has developed in a recipient of these products. A risk assessment carried out in the UK suggested that, on worst case assumptions, some plasma products could be associated with an additional risk of developing vCJD in relation to the background population risk through exposure to BSE and since 1999 plasma for the production of plasma products has been imported to the UK from other countries. The risks from plasma products are probably much lower than the risks from transfusion of labile blood components.

The TMER study has provided evidence that vCJD is transmissible through blood transfusion. The implications for public health have led to measures to minimize the risk from blood transfusion [5] and plasma products derived from cases incubating vCJD and many of these actions were taken years in advance of the evidence for transfusion transmission, both in the UK and many other countries. Although there is uncertainty about the potential for transfusion transmission of vCJD to lead to a self-sustaining epidemic, the introduction of a policy of deferring transfusion recipients as blood donors in the UK has minimized this possibility. The identification of vCJD cases with a history of blood donation in France, Ireland, Spain and Saudi Arabia indicates that this issue has an international dimension. Although the population prevalence of vCJD infection is almost certainly highest in the UK, the identification of secondary transmission of vCJD underlines the importance of international surveillance systems both for human and animal prion diseases.

Acknowledgements

This paper is based on a full review of the TMER study, which has been published in Vox Sanguinis [6]. I am grateful to my colleagues in the TMER study: Professor Robert Will and Ms. Jan MacKenzie (NCDSU) and Dr. Charlotte Llewelyn (National Blood Service), and to our colleagues at the NCJDSU for their support for this project. The study would not have been possible without the collaboration of the National Blood Service (NHS Blood and Transplant), Scottish National Blood Transfusion Service, the Welsh Blood Service and the Northern Ireland Blood Transfusion Service; also the relatives of CJD cases who provided information, and the many clinicians and other staff at UK hospitals and UKBS who have helped to trace records. I thank Dr. Nicky Connor and Dr. Anna Molesworth of the HPA for the data on plasma product batches. The study was funded by the NBS and the Department of Health.

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識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2007 年 8 月 10 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			研究報告の公表状況	Detection of miss-folded prion protein in blood with conformationally sensitive peptides. Pan, T. et al., Transfusion, 47, 1418-1425 (2007).	公表国 米国	
販売名（企業名）						

研究報告の概要

折りたたみ異常プリオン蛋白（PrP^{TSE}）の存在下においてαヘリックスからβシートへの構造の配座変換を行う、高度に保存されたプリオン蛋白（PrP）のN末端領域に由来する小規模なフルオロフォア標識ペプチドの使用について報告した。これは、ペプチドモノマーと凝集ポリマーの区別を放射蛍光波長を変化により可能とする。また、配座の変化は他のペプチド分子に伝播され、それによってシグナル増幅が起こる。著者らは、このPrP^{TSE}の診断的（MDP）アッセイは脳ホモジネート及び伝達性海綿状脳症（TSE）感染マウスの血漿プール内に存在するPrP^{TSE}を特異的に検出可能であるが、正常なマウス又はPrPノックアウトマウスの検体を検討した時にはシグナルは得られなかったことを示している。これらの結果はウェスタンブロット法に完全に一致する。感度閾値は約 1 感染用量／mLと推定される。血漿又は血清に対して適用した場合には、MPDアッセイは未感染の対照と比較して、種々の実験TSE感染の検体と自然TSE感染の検体を区別した。したがって、本アッセイは、ヒト及び動物の双方においてプリオン病の前臨床診断及び臨床診断にあたり有用であると考えられる。

使用上の注意記載状況・その他参考事項等

BYL-2007-0291

報告企業の意見	今後の対応
ここで説明されている折りたたみ異常蛋白質の診断的（MPD）アッセイは PrP の立体配座の変化を検出する非常に有用なアッセイである。著者らは散発性クロイツフェルト・ヤコブ病患者の血漿検体を試験し、その検体が最高で 20 年以上も凍結されていたものであるにもかかわらず、対象となる健康者からの血漿検体と比べ統計学的有意差が認められた。本試験は将来非常に有用となり得る。	現時点で新たな安全対策上の措置を講じる必要はないと考える。引き続き関連情報、特にスケールアップした際の検出可能性についての収集に努める。

Detection of misfolded prion protein in blood with conformationally sensitive peptides

Tao Pan, Jasmeet Sethi, Craig Nelsen, Alan Rudolph, Larisa Cervenakova, Paul Brown, and Cindy S. Orser

BACKGROUND: The long-standing goal of a preclinical diagnostic test for transmissible spongiform encephalopathy (TSE) has recently become urgent because of the discovery that humans with variant Creutzfeldt-Jakob disease can transmit disease via blood transfusions.

STUDY DESIGN AND METHODS: The misfolded protein diagnostic (MPD) assay employs a pyrene-labeled palindromic sequence of prion peptides that undergoes a cascade of coil to β-sheet conversion in the presence of the misfolded prion protein (PrP^{TSE}). The ability of the assay to detect PrP^{TSE} in brain, serum, and plasma was tested. The basic protocol involved a several-hour incubation of 200-µL sample volumes with the peptide reagent in 96-well plates, after which fluorescence was monitored by a fluorescence plate reader with an excitation wavelength of 350 nm and emission scanning wavelength range of 365 to 600 nm.

RESULTS: Target specificity for PrP^{TSE} was documented by correlation of assay signal with Western blot signals in brain tissue from TSE-infected, normal, and knockout mice and negative assay signals by use of reagents with different peptide sequences. When applied to plasma or serum, the assay discriminated between samples from a variety of experimental and natural TSE infections compared to uninfected controls, with a sensitivity threshold of approximately 1 infectious dose per mL in pooled plasma from TSE-infected mice.

CONCLUSIONS: The MPD assay is a sensitive and specific test for the detection of PrP^{TSE} that may be useful in both preclinical and clinical diagnosis of TSE diseases of animals and humans.

Prions are normal host proteins that may also exist in a misfolded proteinase-resistant conformation (PrP^{TSE}) responsible for disease in animals and humans.[1] The considerable public health consequence of these diseases, which include bovine spongiform encephalopathy, sheep scrapie, chronic wasting disease in deer and elk, and Creutzfeldt-Jakob disease in humans, has led to significant efforts to develop methods of preclinical detection. The transmissibility of transmissible spongiform encephalopathy (TSE) via blood has been established in animal models for Gerstmann-Sträussler-Scheinker (GSS) disease and variant Creutzfeldt-Jakob disease (vCJD) in mice,[2-5] scrapie in hamsters,[6] and bovine spongiform encephalopathy in sheep.[7,8] Blood is also infectious in endemic sheep scrapie[9] and in human infections with vCJD.[10-12]

There are several challenges to detecting PrP^{TSE} in blood. The level of PrP^{TSE}, especially early in disease, is below the detection limits of standard immunoassay methods, and the coexistence of a large amount of normal cellular form of the protein (PrP^{C}) compromises detection

ABBREVIATIONS: CTI = C-terminal inversion; GSS = Gerstmann-Sträussler-Scheinker; ID(s) = infectious dose(s); MPD = misfolded protein diagnostic; PrP = prion protein; PrP^{C} = cellular form of prion protein; PrP/KO mouse = PrP knockout mouse; PrP^{TSE} = misfolded form of prion protein; vCJD = variant Creutzfeldt-Jakob disease.

From Adlyfe Inc., Rockville; the American Red Cross Holland Laboratory, Rockville; and 7815 Exeter Road, Bethesda, Maryland.

Address reprint requests to: Dr Cindy Orser, Adlyfe Inc., 9430 Key West Avenue, Rockville, MD 20850; e-mail: corser@adlyfe.com.

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specificity without the use of proteinase enzyme to digest interfering species. PrP^{TSE} does not elicit a humoral immune response in the infected host, preventing detection of a serologic antibody.[13,14] The absence of a foreign nucleic acid component in the infectious particle eliminates the use of PCR; however, immuno–polymerase chain reaction has been used to detect both recombinant PrP and PrP^{TSE} from scrapie-infected brain homogenate at a level 1 million-fold more sensitive than antibody-based detection methods, positioning immuno–polymerase chain reaction as capable of detecting PrP^{TSE} in the preclinical phase of disease in blood.[15]

These challenges have restricted diagnostic tests to the use of postmortem tissue and, in some cases, antemortem lymphoreticular tissue, but there is an urgent need for a preclinical screening test to prevent secondary transmission of vCJD disease from blood donation. We have developed a method of detecting PrP^{TSE} in blood that utilizes a conformationally sensitive pyrene-labeled peptide from the N-terminal region of PrP that contains conserved amino acid sequences[16] (Pronucleon ligands).

The structural features of the peptide have been previously reported, including circular dichroism studies documenting the thermodynamic conditions for its transition from a coiled structure to a β-sheet structure that occurs in the presence of PrP^{TSE}.[17] This conformational change induces an excimeric signal from the conjugated pyrenes when the conformational change to β-sheet brings the pyrene pair into close proximity. The conformational change is propagated to other peptide molecules, serving to amplify the signal for the presence of PrP^{TSE}. We have previously shown that these peptides can detect PrP^{TSE} in brain during the preclinical and clinical stages of disease in hamsters infected with the 263K strain of scrapie.[16] We here extended these studies to the detection of PrP^{TSE} in the blood of animals and humans with various forms of TSE.

MATERIALS AND METHODS

PrP^{TSE} peptides and reagents

The misfolded protein diagnostic (MPD) peptide, formerly referred to as 33-H1,[16] was used for the detection and amplification of PrP^{TSE} in all tissue and plasma samples in this report. Two additional peptides were also used to evaluate specificity of the reaction: a C-terminal inversion (CTI) and a randomly scrambled version of the MPD peptide The CTI peptide sequence was VVAGAAAA-GAVHKLVVAGAAAAGAVHKLNTKPK, and the scrambled sequence was LKVAHVAPAGGAKGGAHLAAVAVKAAT-KVAAVNK. All peptides had pyrene conjugated to both ends. The peptides were synthesized with solid-support FMOC synthesis methods and provided as a desiccated powder with greater than 95 percent purity as determined by amino acid sequence and mass spectroscopy (New England Peptide, Gardner, MA). The peptides were aliquoted from a dry powder and stored in dimethyl sulfoxide (or hexafluoroisopropanol) at –80°C as a 1 mg per mL stock reagent until use in the PrP^{TSE} assay.

The stock peptide reagent was diluted to a 2 μmol per L solution in 50 percent trifluoroethanol (Sigma Aldrich, St Louis, MO), 50 percent 10 mmol per L Tris, pH 7.4, and allowed to equilibrate for 1 hour at room temperature before the addition of the test sample. The trifluoroethanol and peptide concentrations were previously optimized to provide the maximum separation between background peptide misfolding and self-aggregation and substrate-induced conformational change in the peptide as well as to minimize fluorescence quenching.[17] Test samples were either used as such or partially purified with methods described for a given experiment. Twenty-microliter sample volumes were added to 180 μL of the equilibrated peptide solution. Reactions were allowed to incubate at ambient temperature with fluorescence readings taken at 1, 3, and 5 hours. A sample containing only the peptide was included in each test run as a control on peptide stability and self-induced conformational change during the incubation. For the standardized MPD reaction, the signal of the peptide only control reaction was subtracted from the signals of test sample reactions. Reactions were monitored in 96-well plates in a fluorescent plate reader (Safire II, Tecan, Mannedorf, Switzerland) with an excitation wavelength of 350 nm and emission scanning from 365 to 600 nm. The resulting PrP^{TSE} signals were analyzed as the natural log (ln) of the integrated excimeric fluorescence from 430 to 530 nm in all cases and for Fig. 4 as the ratio of pyrene $excimeric_{max}$ fluorescence to pyrene $monomeric_{max}$ fluorescence as well. Integrated peak values were from 370 to 385 nm and from 430 to 530 nm that correspond to monomer and excimer emission, respectively.

GSS murine model

Brains from a normal Swiss mouse, a PrP knockout (PrP/KO) mouse (gift of C. Weissman to L. Cervenakova) or a GSS (Fukuoka-1 strain)-infected mouse were homogenized in 10 percent phosphate-buffered saline (PBS). The brain homogenates were analyzed by Western blot according to previously published methods[5] and precipitated with phosphotungstic acid according to a previously published method for evaluation in the MPD assay.[16] For Western blot analysis, 10 μL of 1 percent GSS or 10 percent Swiss or PrP/KO brain homogenates were mixed with 10 μL of Laemmli sample buffer containing 5 percent β-mercaptoethanol, heated at 95°C for 10 minutes, and all loaded into 1-mm, 10-well, 4 to 12 percent Bis-Tris gel (Invitrogen, Carlsbad, CA), which after electrophoresis was transferred onto a 0.45-μm nitrocellulose membrane (Invitrogen).

TABLE 1. Specimens analyzed in the MPD assay reported in this study

Species	Number of animals: Infected	Number of animals: Control	Route of inoculation	Infecting strain	Tissue
Sheep	13	1 pool of 25		Endemic	Serum
Mouse	1 pool of 75	1 pool of 12 and 1 pool of 5 knockout	IC*	Fukuoka-1	Plasma
Mouse	1	1 and 1 pool of 5 knockout	IC	Fukuoka-1	Brain
Human	5	5		Sporadic	Plasma
Monkey	8	4	IC	Human sporadic CJD	Plasma

* IC = intracerebral.

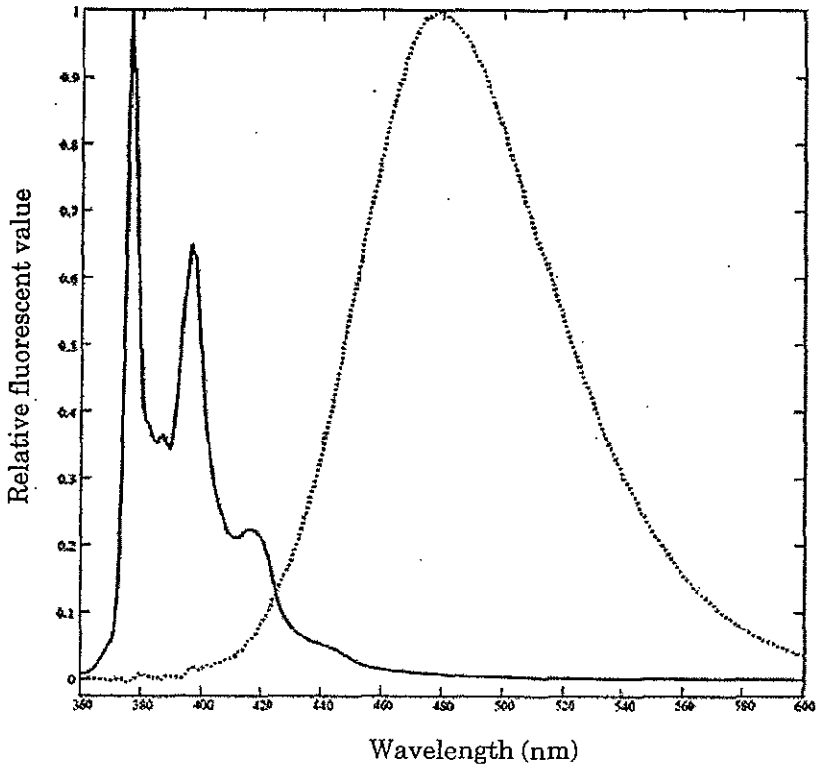

Fig. 1. Association of conformational change with fluorescence. Conformational change in the peptide is associated with a shift in fluorescence intensity from pyrene monomer to pyrene excimer emission. Monomeric pyrene molecule emission is characterized by the solid line spectrum with a maximum from 375 to 385 nm, whereas the excimeric form of pyrene, associated with β-sheet conformation, is portrayed by the dashed line spectrum with a maximum from 460 to 500 nm. The *x*-axis is wavelength (nm) and the *y*-axis is an arbitrary fluorescence scale.

Western blots were performed with primary antibody 6D11 (Signet Laboratories, Dedham, MA) at a 1:10,000 dilution, peroxidase-labeled goat anti-mouse immunoglobulin G (KPL, Gaithersburg, MD) at a dilution of 1:20,000, and chemiluminescent substrate (Super Signal West Pico, Pierce, Rockford, IL). The MPD assay used 10 µL of a 1:1,000 dilution of brain homogenate incubated with one of three different peptides, the PrP^{TSE} MPD peptide, the CTI, or a scrambled version of the peptide. Samples were treated with 50 µg per mL (final concentration) proteinase K (Sigma Aldrich) at 37°C for 1 hour, terminated by the addition of phenylmethylsulfonyl fluoride (Sigma) at a final concentration of 3 mmol per L.

Blood was obtained from 75 symptomatic mice that were euthanized at an advanced stage of disease 18 weeks after intracerebral inoculation with the Fukuoka-1 strain of GSS.[4] The blood was collected in citrate phosphate dextrose anticoagulant and pooled, and the plasma was separated and stored at −70°C. The infectious titer had previously been determined by bioassay to be 10 infectious doses (IDs) per mL. The MPD assay was performed on serial dilutions of an aliquot that had been stored at −70°C for 8 years, the small volume of which prevented replicate testing for statistical analysis. The MPD assay reactions were then fractionated on 2-mL Sepharose 2B (Sigma) columns to separate plasma components by size eluting with 0.5× PBS and collecting 100-µL fractions. Twenty-microliter aliquots of each fraction were analyzed in the MPD assay with preequilibrated peptide solution as described above. Two fractions showing the best discrimination of normal from infected were chosen for statistical evaluation.

Sporadic CJD primate model

Pooled brain homogenates were prepared from two human patients with sporadic CJD, and 0.1-mL volumes were inoculated intracerebrally at 10^{-1} or 10^{-3} dilutions into the left frontal cortex of eight squirrel monkeys.[18] Animals were kept under continuous clinical surveillance and regularly underwent standardized behavioral testing. The time to initial clinical signs ranged from 20.5 to 23 months after inoculation, with clinical signs lasting from 1 week to 7 months. Postmortem examinations revealed typical patterns of spongiform change and PrP^{TSE}

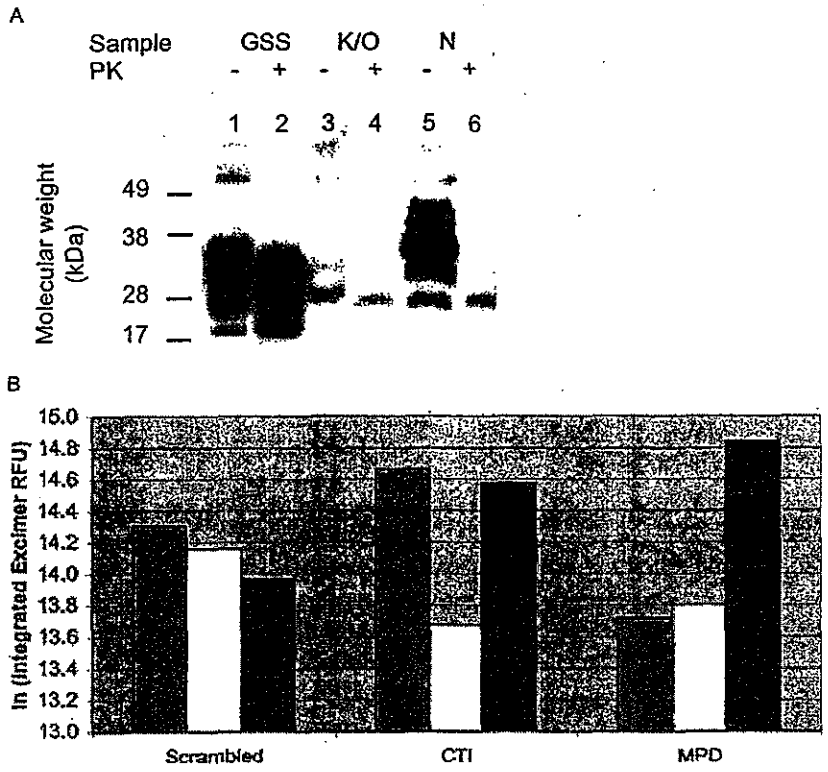

Fig. 2. MPD assay specificity. (A) Western blots of brain homogenates from a GSS-infected mouse, a PrP/KO mouse, and a normal mouse. Lanes 1 and 2 = GSS disease; Lanes 3 and 4 = PrP/KO; Lanes 5 and 6 = normal (N). Lanes 2, 4, and 6 = proteinase K (PK)-treated; Lanes 1, 3, and 5 = no PK treatment. See Materials and Methods for protocol details. (B) Brain homogenates from knockout (□), normal (▨), or GSS-infected (■) mice were evaluated with MPD, CTI, or scrambled peptides following the protocol described under Materials and Methods. Values are presented as the ln (integrated relative fluorescent value [RFU] for the excimer).

distribution similar to those seen in humans. Four uninoculated control animals were housed under identical conditions and bled at the same time as the inoculated animals. Fifty-microliter plasma aliquots from terminal bleeds were fractionated on 2-mL Sepharose 2B (Sigma) columns to separate plasma components by size eluting with 0.5× PBS and collecting 100-µL fractions. Twenty-microliter aliquots of the resulting fractions were analyzed in the MPD assay with preequilibrated peptide solution as described above under Materials and Methods.

Sporadic CJD in humans

Plasma samples obtained from five symptomatic neuropathologically verified and experimentally transmitted cases and five healthy individuals were stored at –70°C for periods of 10 to 20 years. One-hundred-microliter aliquots were fractionated on 2-mL Sepharose 2B (Sigma) columns to separate plasma components by size eluting with 0.5× PBS and collecting 100-µL fractions. Twenty-microliter aliquots of fractions were analyzed in the MPD assay with preequilibrated peptide solution as described above.

Endemic scrapie in sheep

Thirteen serum samples from neuropathologically and immunohistochemically confirmed sheep were obtained from the USDA Laboratory in Ames, Iowa. A pool of 25 normal sheep serum samples was purchased from Rockland Immunochemicals Inc. (Gilbertville, PA) and processed as the scrapie-free control. Serum glycoproteins were precipitated with phosphotungstic acid with a modification of a previously published method.[15] Two-hundred-microliter volumes were mixed by vortexing with an equal volume of 2× extraction buffer (20 mmol/L Tris, pH 7.4, 0.2 mol/L NaCl, 1.0% Igepal CA-360, 2% deoxycholic acid, 0.02% NaAz). Phosphotungstic acid (4%) was then added to a final concentration of 0.3 percent and the mixture was again vortexed, incubated for 1 hour with rocking, followed by centrifugation at 14,000 × g for 30 minutes. The resulting pellet was resuspended in PBS, and 20-µL aliquots were analyzed in the MPD assay as described above.

Statistical analysis

When adequate volumes permitted, samples were run in triplicate and standard deviations were calculated. Control samples from healthy animals or humans were run as a qualitative comparison in all cases. Peptide reagents (in the absence of samples) were routinely run in triplicate as a control for background peptide dynamics. Statistical analysis was used to discriminate positive from negative values whenever possible, with a t test or the binomial test when comparing a control pool to individual infected samples in the case of the sheep serum samples to generate the p values given with the figures.

RESULTS

Table 1 summarizes the samples evaluated in this study that spans a period of both sample processing and MPD assay method development as well as data analysis and presentation across a diverse set of blood samples.

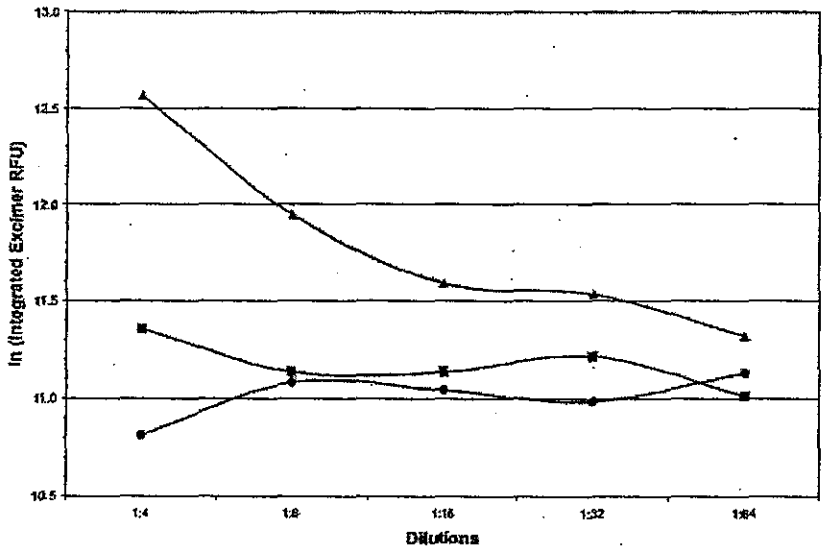

Fig. 3. MPD assay sensitivity. A dilution series of a previously titered terminal plasma pool from mice experimentally infected with the Fukuoka-1 strain of GSS (▲), a normal mouse plasma pool (■), and plasma from a PrP/KO mouse (●) were evaluated. Dilutions were made in PBS and read in a 200-µL reaction volume. The data are presented as the ln (integrated relative fluorescent value [RFU] for the excimer).

Fluorescence measurement of conformational change

Fluorophore-labeled peptide reagents were used to follow conformational change induced by the presence of sequence-specific misfolded PrP^{TSE} molecules. Figure 1 demonstrates the two distinct fluorescence spectra possible for the double pyrene-labeled peptides. Integrated peak values from 370 to 385 nm and from 430 to 530 nm correspond to monomer and excimer emission, respectively. The ratio of relative excimer fluorescence to relative monomer fluorescence emission provides a measurement of the presence of β-sheet-rich PrP^{TSE} target substrate.

Specificity of the MPD assay

To establish that the MPD assay detects PrP^{TSE}, we compared the results of MPD and Western blot assays of brain tissue from a GSS-infected mouse, a normal mouse, and a PrP/KO mouse. Western blots of these samples after treatment with proteinase K yielded the expected downward band shift of PrP^{TSE} in the GSS-infected brain and the absence of PrP^{C} bands in the normal and knockout specimens (Fig. 2A).

We also compared the MPD peptide reagent on the same three mouse brain homogenates with two MPD peptide variants, a scrambled version that produced no distinction across the three murine samples, and a CTI MPD peptide that distinguished both the normal and the GSS brain tissue from the KO mouse, but not the normal mouse, illustrating the importance of the correct structural sequence of the MPD peptide in distinguishing PrP^{TSE} from PrP^{C}. Only the MPD assay peptide, with both the proper sequence and the conformationally sensitive structure, was able to distinguish between the infected mouse brain sample and both uninfected samples (Fig. 2B).

Sensitivity of the MPD assay

Plasma from a pool of terminally ill GSS-infected mice was compared to plasma pools from normal and PrP/KO mice in a parallel series of twofold dilutions. The infected mouse plasma had been previously bioassayed and shown to contain 10 IDs per mL.[5] The positive signal at a 1:8 dilution and negative signal at a 1:16 dilution indicates a threshold of test sensitivity in the range of 1 ID per mL (Fig. 3).

Detection of PrP^{TSE} in blood: sporadic CJD primate model

A set of 12 coded plasma samples from the terminal bleeds from squirrel monkeys inoculated with a strain of human sporadic CJD and from uninoculated control monkeys was enriched in a fractionation protocol prior to evaluation in the MPD assay. Analysis of the data set with the natural log of the integrated excimeric peak (as done with all other experiments) is shown in Fig. 4A. Three of the 12 coded samples had recognizably "high" signals. A different analytic approach comparing the ratio of the excimeric signal to the monomeric signal in each of the different fractions revealed two fractions that gave a much cleaner separation of the samples into two groups: 8 samples with higher signals and 4 samples with lower signals. Based on signals recorded from one of these fractions, the two groups were scored as positive and negative before the sample identifications were "unblinded." The results showed an excellent correlation of high and low signals in inoculated and uninoculated animals, respectively, as shown in Fig. 4B. Statistical analysis yielded a p value of 0.002. This alternative data analysis was more appropriate for the protocol used in this experiment, which separated molecules present in the plasma samples, including the PrP^{TSE}, before introduction of the MPD peptide. The resulting fluorescence spectra differed from those produced by unfractionated samples that benefit from comparison of the excimeric peak to the monomeric peak.

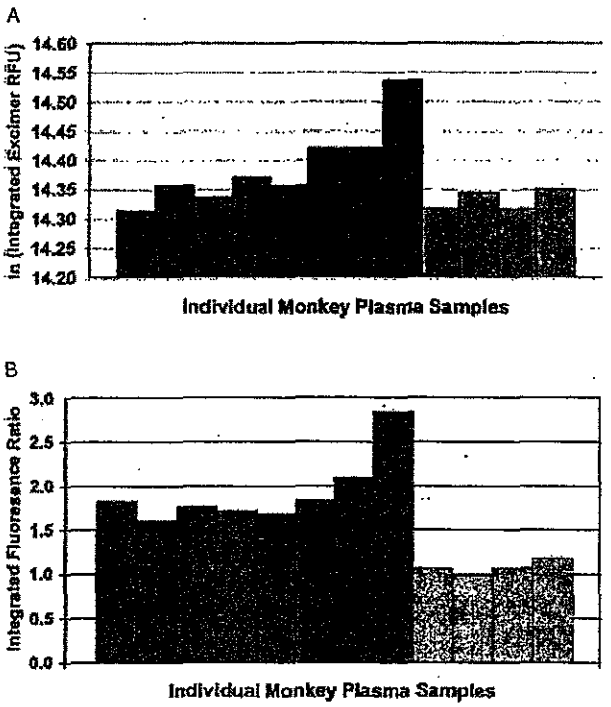

Fig. 4. MPD assay of sporadic CJD–infected primate plasma (blinded study). Coded terminal plasma samples from squirrel monkeys either inoculated with a human strain of GSS or uninoculated were evaluated. Thirteen samples fell into two distinct groups that upon breaking the code were revealed to correspond to infected (■) and uninfected (▦) animals. Data are presented in two formats: (A) Values are ln (integrated relative fluorescent value [RFU] for the excimer); (B) values are presented as the ratio of the integrated excimeric RFU to the monomeric RFUs (p = 0.002 by t test).

Detection of PrP^{TSE} in blood: endogenous infection

Human sporadic CJD. Blinded tests of a small number of plasma samples from human sporadic CJD patients showed modest but clearly increased signals from four of five CJD patients and a high signal from one of the five patients; all control samples were negative (Fig. 5). Statistical analysis of the patient and control groups yielded a p value of 0.03.

Sheep with natural scrapie. Thirteen serum samples from symptomatic sheep that on autopsy were histopathologically and immunohistochemically confirmed to have scrapie were compared to a pool of 25 serum samples from scrapie-free sheep (Fig. 6). Statistical comparison of the scrapie and control group yielded a p value of less than 0.001 with a binomial statistical test indicating that all 13 of the serum samples from scrapie animals were above the population mean of the pooled control serum sample. Individual control animal serum samples from the pool were not available to test.

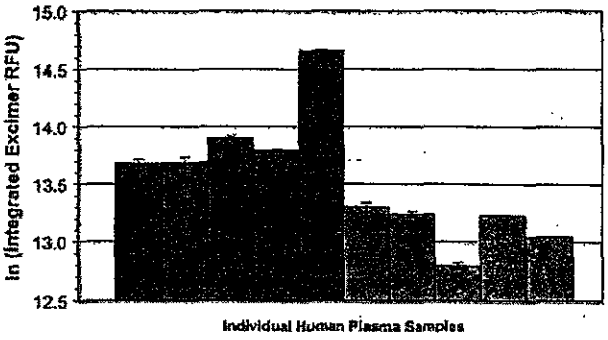

Fig. 5. MPD assay of human sporadic CJD plasma samples (blinded study). Five plasma samples from clinical sporadic CJD patients (■) and five normal human plasma controls (▦) were evaluated in the MPD assay. The data are presented as ln (integrated relative fluorescent value [RFU] for the excimer) (p = 0.03 with a two-sample t test).

DISCUSSION

One of the few undisputed facts about TSE is that the pathologic process depends on an α-helical coil to β-sheet molecular transformation of the normal cellular prion protein (PrP) into a proteinase-resistant amyloidogenic molecule (PrP^{TSE}), leading to deposition and accumulation of amyloid within the central nervous system and, to a lesser degree, lymphoreticular and other tissues. A variety of diagnostic tests have therefore been developed to detect the presence of PrP^{TSE} in these tissues and in circulating blood. We have used a small fluorophore-containing peptide, with homologous sequence specificity to a highly conserved region of PrP that is induced to undergo a similar conformational change in the presence of PrP^{TSE}. We present data showing that the MPD assay specifically detects PrP^{TSE}, with a threshold of sensitivity of approximately 1 ID per mL, and have applied the assay to the detection of PrP^{TSE} in the blood of experimentally infected mice and primates, endemic scrapie in sheep, and sporadic CJD in humans.

The signal generated in the assay is directly related to pyrene excimer formation induced by the peptide conformational change in the peptide. The thermodynamic conditions necessary to hold the peptide in an α-helical conformation, stabilizing the peptide monomer before addition of PrP^{TSE} as a reaction initiator, were the subject of previous studies and are important to the performance of the assay.[17] Monitoring of peptide activity through the use of synthetic substrates to induce maximal excimer formation is also critical to demonstrating the ability of the peptides to undergo a detectable conformational change induced by the presence of PrP^{TSE}.

Binding of tissue PrP^{TSE} to the MPD peptide, as well as peptide-to-peptide association, contribute to signal generation.[16] We have also considered the possibility that

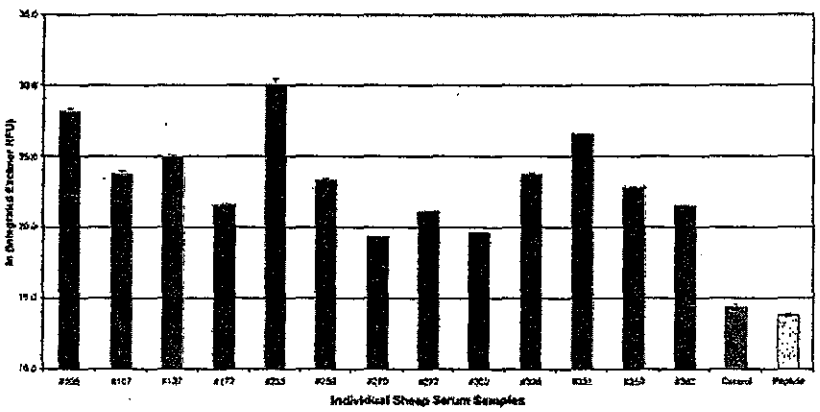

Fig. 6. MPD assay of scrapie-infected sheep serum. Values represent the mean of three replicated readings of individual scrapie sheep serum samples (■), a pool of 25 normal sheep serums (▨), and a MPD peptide only control (■); data are presented as ln integrated relative fluorescent value (RFU) for the excimer ($p < 0.001$ with a binomial test).

excimer formation could arise as a result of conformational changes induced by the presence of other β-sheet proteins or other prevalent proteins in blood, such as bovine serum albumin. The monitoring of peptide excimer formation in control samples indicates that pyrene excimer formation observed in the reaction was specific to PrP^{TSE}.

We previously reported a time point study of brain tissue from scrapie-infected hamsters in which the MPD signal became positive as early as 3 weeks after infection, well before the appearance of Western blot positivity,[16] a finding that invites similar evaluation of blood during the preclinical phase of TSE. Several other laboratories are currently working on methods that may be sufficiently sensitive and specific to detect the very low levels of PrP^{TSE} circulating in blood during the clinical, and in some cases, preclinical stages of TSE.[19-21] Different strategies have been devised that either concentrate or amplify the amount of PrP^{TSE} to detectable levels. These may involve the use of a "capture" antibody or chemical ligand that accomplishes enrichment of the molecular target.[22,23] Some methods use proteinase K to digest PrP^{C} or a denaturing agent that unfolds the PrP^{TSE} and exposes epitopes that were hidden within the interior of the unfolded molecular aggregate.[24] Other laboratories make use of an antibody that is specific for PrP^{TSE} without a preceding digestion or denaturation step.[24,25]

One interesting method utilizes the repeated sonication and amplification of PrP^{TSE} in blood with the addition of PrP^{C} substrate from brain tissue.[26-28] Although the assay method has succeeded in quantifying PrP^{TSE} in blood, it has the practical drawbacks of long cycle times of sonication and amplification (days) and amplification of infectious PrP^{TSE}. To the best of our knowledge, the MPD assay is unique in not using an antibody at any stage of the test, relying on a reactive synthetic peptide for both specificity and amplification. All of the published PrP^{TSE} detection methods have achieved successful detection of PrP^{TSE} "spikes" into blood, some have achieved success with blood collected from the clinical phase of disease in either animal or human TSEs, and a few have extended this success to include preclinical detection in animals. None has so far been able to reach the ultimate goal of identifying preclinical infection in humans. The MPD test has been previously shown to discriminate TSE-infected brain tissue from normal brain tissue,[15] and in this article we have expanded the spectrum of tissues to include blood in a variety of natural and experimental TSE infections.

The performance requirements for a practical method of blood detection for PrP^{TSE} depend on the diagnostic goal: whether as a confirmatory test of the clinical diagnosis in symptomatic animals and humans suspected of having TSE or as a test to identify infection during the preclinical phase of disease, that is, as a diagnostic screening test. A confirmatory diagnostic test requires a high degree of sensitivity, whereas a screening test mandates a high degree of specificity because of the need for an absolute minimum number of false-positive samples in the very large number of samples that would be screened.

Much recent research has focused on screening test applications, both as a tool to identify scrapie infections in animals that may then be culled from flocks to prevent continued spreading of disease (perhaps eventually also to identify chronic wasting disease of deer and elk) and as a way of identifying infected but apparently healthy blood donors to avoid secondary iatrogenic transmissions. We continue our efforts to optimize the conditions of the MPD assay to widen the margin of significance between positive and negative test values and to improve reproducibility and "packaging" as a practical test for use in blood collection centers.

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from Susan Gibson, DVM University of South Alabama, AL; and Thomas R. Kreil, PhD, Baxter Bioscience, Vienna, Austria.

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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

問題点（ドイツでのバベシア症の感染報告）
バベシア症は動物に共通した感染症であり、欧州において新興のダニ媒介人獣共通感染症として注目されている。
この論文は、ドイツで発症したヒトバベシア症の最初の報告である。
患者は 63 歳の男性のドイツ人で、結節性リンパ球優位性ホジキンリンパ腫が再発し脾臓摘出術を受けていた。
患者がリツキシマブ治療後、貧血とヘモグロビン尿による暗色尿のため入院した。
末梢血液塗抹標本（ギムザ染色）による診断で、梨状で変形体様の寄生虫が赤血球内で確認されたが、B. microti と B. divergenes の IgM、IgG 特異抗体は陰性であった。
PCR 法により 18S-rRNA をコードする rDNA 領域を増幅して、増幅された DNA を比較すると、Herwaldt らにより EU1 に指定されたバベシアと 99%以上のホモロジーを有していた。
患者はダニに刺された記憶がなく、入院前に輸血歴もない。
人獣共通感染能のあるバベシア症が特有な地方において、慢性的な感染者の輸血により感染することが知られている。

報告企業の意見	今後の対応
バベシア症は赤血球内にバベシア原虫が寄生するため発症するが、血漿分画製剤は血漿を原材料にしているため感染はないと考えられる。万一原料血漿に混入しても、製造工程で除去できると考えている。	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。

使用上の注意記載状況・その他参考事項等

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First case of human babesiosis in Germany – Clinical presentation and molecular characterisation of the pathogen

Katja Häselbarth[a], Astrid M. Tenter[b], Volker Brade[c], Gerhard Krieger[a], Klaus-Peter Hunfeld[c,*]

[a]*Department of Internal Medicine II, Hegau-Bodensee-Medical Center, Singen, Germany*
[b]*Department of Parasitology, School of Veterinary Medicine, Hannover, Germany*
[c]*Institute of Microbiology and Infection Control, University Hospital of Frankfurt/Main, Paul-Ehrlich Street 40, D-60596 Frankfurt/Main, Germany*

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Abstract

Babesiosis is a common infection of animals and is gaining increasing attention as an emerging tick-borne zoonosis of humans in Europe. Here we report on the first case of human babesiosis in Germany in a 63-year-old splenectomised German patient with a relapse of nodular lymphocyte-predominant Hodgkin's lymphoma. After treatment with a chimeric anti-CD20 antibody preparation (Rituximab), the patient was hospitalised because of anaemia and dark urine from haemoglobinuria. Presumptive diagnosis of babesiosis was made based on piriform parasitic erythrocytic inclusions in peripheral blood smears and confirmed by *Babesia*-specific 18S rDNA PCR. Sequence analysis revealed a >99% homology of the amplicon with the recently described EU1 organism clustering within the *Babesia divergens/Babesia odocoilei* complex. Despite treatment with quinine and clindamycin the patient relapsed and developed chronic parasitaemia requiring re-treatment and long-term maintenance therapy with atovaquone before he eventually seroconverted and the parasite was cleared. Our findings suggest that human babesiosis occurs in Germany and can take a chronic course in immunocompromised individuals.
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Keywords: Babesiosis; Germany; Immunocompromised patient; *Babesia* EU1

Introduction

Tick-borne infections are among the most important vector-borne infections in the northern hemisphere (Hunfeld and Brade, 2004). New infections have emerged all over Europe and the incidence of such diseases is rising steadily. Moreover, changes in the distribution and frequency of tick-borne diseases may be among the first impacts of global climatic changes on human health (Skarphedinsson et al., 2005). Babesiosis, which is caused by intraerythrocytic parasites of the protozoan genus *Babesia*, is one of the more common diseases of free-living animals worldwide and is gaining increasing attention as an emerging tick-borne zoonosis in humans (Herwaldt et al., 2003; Homer et al., 2000). The parasites are named after the Romanian scientist Victor Babes, who first identified pear-shaped, *Plasmodium*-like protozoan parasites as the causative agents of

*Corresponding author. Tel.: +49 69 6301 6441; fax: +49 69 6301 5767.
E-mail address: K.Hunfeld@em.uni-frankfurt.de (K.-P. Hunfeld).

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febrile haemoglubinuria in cattle (Babes, 1888). Since the late 1950s, two species of *Babesia* in particular, the cattle species *Babesia divergens* in Europe and the rodent species *Babesia microti* in North America, have been shown to cause a significant number of infections in humans (Homer et al., 2000; Hunfeld and Brade, 2004). The frequent reports of human babesiosis cases in North America, however, are in striking contrast to only sporadic reports of the disease in Europe and other parts of the world. However, in many non-American regions, microbiological investigations and awareness of the clinical presentation of babesiosis in humans lag far behind those in the USA and Canada. In Germany, *Ixodes ricinus* is regarded the most important vector for tick-borne diseases in humans and, although it is not known for certain, there is circumstantial evidence that *I. ricinus* transmits at least *B. divergens* and *B. microti* to humans (Gray, 2006; Hunfeld and Brade, 2004). This tick is abundant throughout the country but no indigenous cases of human babesiosis have been documented in Germany so far. Here we report on detection and clinical management of the first autochthonous case of human babesiosis in Germany.

Materials and methods

Serological testing for *Babesia* spp.

Serological testing for specific IgG and IgM antibodies was performed using well-evaluated indirect immuno-fluorescence assays (IFA) for *B. microti* and *B. divergens* as described recently in more detail (Hunfeld et al., 2002). For detection and semiquantification of *B. microti*-specific antibodies, golden hamster erythrocytes infected with the *B. microti* GI strain, which was originally isolated from a human patient from the east coast of the United States, were used. For detection of antibodies to *B. divergens*, IFA antigens were prepared from the blood of jirds (*Meriones unguiculatus*) experimentally infected with a *B. divergens* isolate that was originally obtained from naturally infected cattle in northern Germany. All positive sera were titrated in triplicate on different days, and titres are reported as geometric mean titres. In addition, sera that were tested in IgM assays were pre-incubated with rheumatoid factor absorbance reagent (Focus, Cyprus, USA). Measurement of antibodies started at a serum dilution of 1:20 (IgM) or 1:16 (IgG). Sera were then diluted serially in twofold steps to determine end point titres (Hunfeld et al., 2002).

PCR for detection of *B. microti*

DNA extraction was performed by use of commercial DNA extraction kits (High Pure Template PCR Preparation Kit, Roche, Germany) according to the manufacturer's instructions. For the performance of PCR, the Taq DNA Polymerase Kit (Invitrogen, Germany) was applied. PCR was performed according to Persing et al. (1992), with some modifications. Each PCR run included a DNA preparation of the *B. microti* GI strain and a negative control. A separate preparation containing 8 µl DNA extracted from the corresponding clinical sample in addition to 2 µl of positive control DNA was co-amplified in each run to serve as inhibition control. The PCR mixture (100 µl) consisted of 10 µl of purified DNA preparation and 90 µl PCR mix [10 µl PCR buffer (10 × concentration: 200 mM Tris–HCl, pH 8.0, 500 mM KCl), 5 µl $MgCl_2$ (50 mM), 1 µl dNTPs (10 mM), 1 µl Bab1 primer (100 pmol), 1 µl Bab 4 primer (100 pmol), 71.5 µl distilled water, and 0.5 µl Taq DNA polymerase (5 U/µl)]. The amplification reaction was performed on a programmable Gene Amp PCR System 9600 (Perkin Elmer, Germany), with a three-step cycling programme as follows: 1 min of denaturation at 94 °C, 1 min of annealing at 55 °C, and 2 min of extension at 72 °C for a total of 35 cycles. The 238-bp product was then visualised on an ethidium bromide-stained 1.5% agarose gel by UV gel imaging after electophoresis on a Bio-Rad apparatus (Bio-Rad, Germany).

PCR for the detection of *Babesia* spp.

DNA extraction was performed as described above. PCR was performed as outlined by Armstrong et al. (1998), with some modifications. The forward primer PIRO-A and the reverse primer PIRO-B flank 407-, 408-, and 437-bp nucleotide regions of the 18S rRNA gene of *Babesia* spp. Each PCR run included a DNA preparation of the GI strain of *B. microti* and a negative control (water blank). A separate preparation containing 12 µl DNA extracted from the corresponding clinical sample in addition to 3 µl of positive control DNA was co-amplified in each run to serve as inhibition control (Fig. 1). For the PCR, the ELONGase Amplification System (Life Technologies, Germany) was used. The initial PCR mixture (40 µl) consisted of 15 µl of purified DNA preparation, 11 µl mix I [1 µl dNTPs (10 mM), 5 µl primer PIRO-A (100 pmol), and 5 µl primer PIRO-B (100 pmol)], and 14 µl distilled water. This mixture then was filled to a total volume of 100 µl by adding 60 µl of mix II (10 µl buffer A, 10 µl buffer B, 2 µl ELONGase enzyme mix, and 38 µl distilled water).

Thermocycling was performed on a programmable Gene Amp PCR System 9600 (Perkin Elmer, Germany) with a denaturation step of 1 min at 94 °C, followed by 40 cycles each consisting of 40 s at 94 °C, 45 s at 55 °C, and 45 s at 72 °C. The resulting amplification product was visualised as described above.

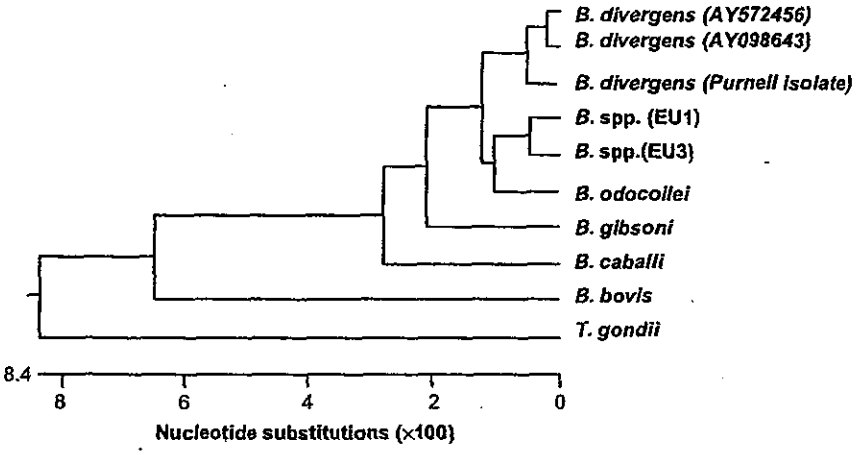

Fig. 1. Phylogenetic tree calculated for the 18S rRNA gene sequences as available from GeneBank for selected *Babesia* spp. and for our newly detected *Babesia* organism (EU3). The tree was computed by using the Clustal method with weighted residue weight table (MEGALINE program, DNASTAR, Lasergene, Germany). The accession numbers for the sequences obtained from GenBank that have been used in the analysis are as follows: *B. bovis*, L19077; *B. caballi*, Z15104; *B. divergens* (Purnell isolate), AY046576; *B. divergens*, AY572456; *B. divergens*, AY098643; *B. gibsoni* (genotype Asia 1), AF175300; *B. odocoilei*, AY046577; *B.* spp. (EU1), AY046575; and *T. gondii*, M64243.

Molecular characterisation of the pathogen and sequencing of PCR products

Amplification of the complete 18S rRNA gene was performed using *Babesia* DNA extracted from the patient's blood and by use of a generic apicomplexan 18S rDNA-specific PCR as described recently in more detail (Herwaldt et al., 2003) yielding a specific product of 1700 base pairs. The sequencing of all PCR amplification products was performed in both directions by use of an "ABI PRISM™ Dye Terminator Cycle Sequencing Ready Reaction Kit" in accordance with the manufacturer's specifications. Products were sequenced by a commercial specialist laboratory (GATC, Stuttgart, Germany). Obtained sequences were analysed using DNASTAR version 4.0 (Lasergene, Germany) and then submitted to GenBank (NIBC) for comparison with known sequences of reference organisms. A phylogenetic tree (Fig. 1) was then calculated for the 18S rRNA gene sequences as available from GenBank for selected *Babesia* spp. and for our newly detected *Babesia* organism (EU3) by using the Clustal method with weighted residue weight table (MEGALINE program, DNASTAR, Lasergene, Germany).

Case report

In October 2005, a 63-year-old German patient, who had undergone splenectomy in 1973 because of Hodgkin's disease, was admitted to the hospital. He suffered from mild anaemia and enlarged iliacal lymph nodes. Subsequently, a relapse of Hodgkin's disease manifesting itself as paragranuloma (stage: IIa, IPI1) was diagnosed based upon histopathology of a lymph node biopsy. Reactivation of the disease was treated with intravenous application of a chimeric anti-CD20 antibody preparation (Rituximab, MabThera) for 4 weeks. In late November, the patient showed progressive weakness, shortness of breath, and lethargy finally resulting in re-hospitalisation on December 4, 2005 because of haemolytic anaemia (erythrocyte count: 2.7 mio/µl; Hb: 8.6 g/dl), elevated serum lactate dehydrogenase (LDH: 3569 U/l) and bilirubin levels (2.6 mg/dl), and dark urine from haemoglobinuria. His body temperature was slightly elevated (37.8 °C; Table 1). A positive direct Coombs test led to the initial diagnosis of autoimmune haemolytic anaemia potentially owing to ongoing Hodgkin's disease. Therefore, treatment with prednisolone 30 mg per os (p.o.) once daily was initiated. However, the patient did not improve clinically and continued to show elevated leukocyte counts (17,000/µl). Moreover, an elevated C-reactive protein of 14.2 mg/dl and procalcitonin levels of 3.1 µg/dl suggested ongoing infection. Forty-eight hours after admission, haematological examination of peripheral blood smears finally revealed parasitic inclusions (parasitaemia: 4%) in erythrocytes (Fig. 2a–c). The patient admitted doing regular outdoor recreation but he had not travelled beyond his home town recently. Suspicion of malaria was ruled out by a negative dip stick antigen test result (Binax Now Malaria®, Inverness Medical GmbH, Germany). On questioning, he did not recall a tick bite in his recent medical history and no blood products had been administered prior to hospital admission. Presumptive diagnosis of babesiosis was established based

Table 1. Summary of important clinical data, laboratory findings, and microbiological test results in a splenectomised German patient with babesiosis

Clinical information	2005		2006								
	December 4 Admission, weakness, haemoglobinuria, shortness of breath	December 6 Start of treatment with quinine and clindamycin	January 3 Follow-up 10 days after conclusion of treatment, clinical improvement	January 20 Relapse of babesiosis, initiation of re-treatment with atovaquone and azithromycin	January 30 Follow-up, clinical improvement	March 15 Follow-up, clinical improvement	April 5 Control visit, stop of azithomycin therapy, maintenance treatment with atovaquone only, clinical improvement	May 17 Follow-up, physical well-being	June 26 Follow-up, seroconversion, physical well-being	August 9 Follow-up, clearance of parasite, physical well-being	September 7 Follow-up, stop of maintenance therapy
Laboratory data											
CRP (mg/dl)	**14.2**	**11.7**	0.6	**6.1**	0.15	0.3	0.3	n.d.	0.1	n.d.	0.1
Erythrocytes (mio./µl)	**2.7**	**2.5**	**3.6**	**3.7**	n.d.	**4.7**	**4.9**	**4.7**	**4.7**	**4.9**	**4.9**
Haemoglobin (g/dl)	**8.6**	**7.6**	**11.7**	**11.4**	n.d	**14.5**	**14.5**	**14.4**	**14.6**	**15.2**	**15.0**
LDH (U/l)	**3569**	**3263**	**337**	**3030**	**519**	**270**	**401**	**286**	**197**	**207**	**197**
Bilirubin (mg/dl)	**2.6**	**2.3**	0.3	**1.7**	0.6	n.d	0.8	0.7	**1**	0.8	n.d.
Microbiology data											
Parasite count	n.d	+ (4%)	+ (<1‰)	+ (3%)	+ (<1‰)	+ (<1‰)	+ (<1‰)	+ (<1‰)	–	–	–
Babesia spp. PCR	n.d	+	+	+	+	+	+	+	±	–	–
IgM-*B. microti*-IFA	n.d	–	–	–	–	–	–	–	**1:20**	**1:20**	**1:20**
IgG-*B. microti*-IFA	n.d.	–	–	–	–	–	–	–	–	–	**1:16**
IgM-*B. divergens*-IFA	n.d.	–	–	–	–	–	–	–	**1:20**	**1:40**	**1:20**
IgG-*B. divergens*-IFA	n.d.	–	–	–	–	–	–	–	–	**1:32**	**1:128**

LDH: Lactate dehydrogenase; n.d.: not determined; (–): negative; (+): positive; ±: borderline; %: per cent; ‰: per mille; abnormal findings are given in bold type.

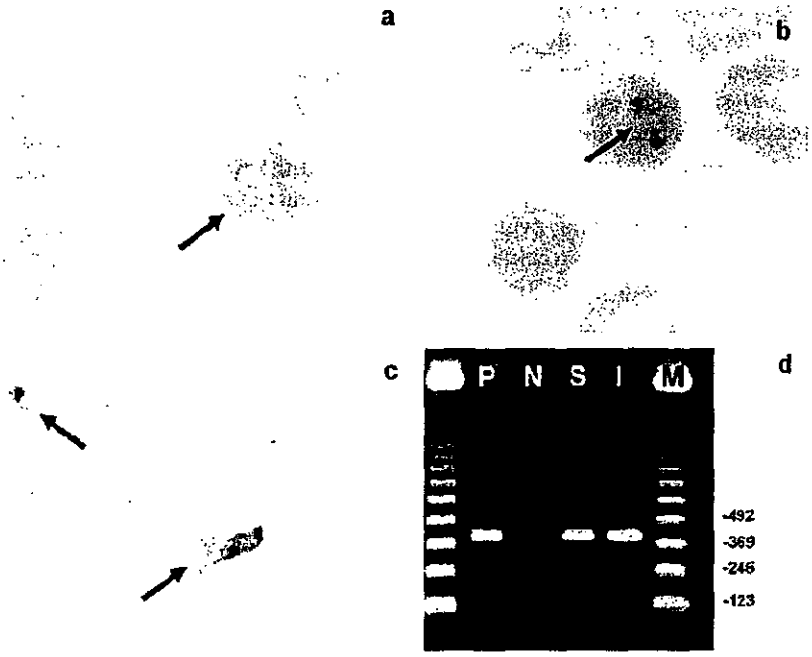

Fig. 2. (a–c) Photomicrographs of *Babesia* (EU3) spp. on a Giemsa-stained smear of peripheral blood showing characteristic piriform inclusions and band forms (trophozoites and merozoites) in infected erythrocytes (denoted by black arrows). Parasites are 1–2.5 μm in diameter. (d) Babesia spp.-specific PCR (PIRO-A and PIRO-B primers) designed for amplification of a highly specific region of the 18S rRNA gene. P: positive control, N: negative control, S: patient sample, I: inhibition control, M: 123-bp marker.

upon the piriform shape (Fig. 2a–c) and the *Plasmodium*-like appearance of the intraerythrocytic parasites. Testing for specific IgM and IgG antibodies against *B. microti* and *B. divergens* by IFA proved negative. The diagnosis was subsequently confirmed by a positive *Babesia*-specific 18S rDNA PCR from EDTA-blood (Fig. 2d). A *B. microti*-specific PCR yielded a negative result. Subsequent sequence analysis of the 18S rRNA gene, amplified by using a generic apicomplexan 18S rDNA-specific PCR (Herwaldt et al., 2003), revealed a 99.7% sequence homology of the PCR product with the recently described EU1 isolate clustering within the *B. divergens/Bodocoilei odocoilei* complex (Fig. 1), and the organism was designated EU3. On December 7, treatment with prednisolone was discontinued and specific anti-parasitic chemotherapy with quinine (650 mg, 3 × /day, p.o.) and clindamycin (600 mg, 3 × / day, p.o.) was initiated. However, the interval between onset of symptoms attributed to babesiosis and the initiation of specific therapy after presumptive diagnosis of the disease was at least 6 days. Attempts to obtain an isolate of the parasite, by injecting several specimens into jirds and mice, were unsuccessful and periodic smears as well as PCR analysis of animal blood remained negative.

The patient initially showed a rapid clinical response to treatment and parasites could no longer be detected in conventional blood smears. After 1 week, however, the application of quinine had to be discontinued because of side effects and treatment was continued with clindamycin only for an additional 10 days. Ten days after therapy, the patient remained well clinically but blood samples became positive for *Babesia* again, both in blood smears and in PCR examination. Four weeks, after the conclusion of treatment, the patient relapsed with recurrent anaemia, sweats, haemoglobinuria, and a parasitaemia of 3% (Table 1). Upon serological examination by IFA the patient remained negative for specific antibodies. Re-treatment was initiated with azithromycin (500 mg, 1 × /day, p. o.) plus atovaquone (750 mg, 2 × /day, p. o.) resulting in rapid clinical improvement of the patient. Nevertheless, direct smears and PCR analysis continued to demonstrate persistent low-level parasitaemia (< 1‰) in follow-up blood samples despite re-treatment for eleven weeks. In addition, the patient remained seronegative upon serological examination for anti-*Babesia* antibodies. To avoid a relapse of babesiosis, atovaquone maintenance therapy was therefore continued for an additional 5 months. The patient continued to improve clinically since February 2006 and laboratory parameters returned to normal levels (Table 1). He finally seroconverted for IgM antibodies (titre: IgM: 20) both, in the *B. microti* and *B. divergens* IFA in late June and

for *B. divergens* IgG antibodies (IgG: 32) in early August. In August 2006, he eventually cleared the parasite as demonstrated in direct blood smears and by negative PCR analysis. Following an additional 4 weeks of atovaquone maintenance therapy, laboratory examinations revealed increasing IgG antibody titres (*B. divergens* IFA titre: IgM: 20, IgG: 128; *B. microti* IFA titre: IgM: 20, IgG: 16) and no evidence for persistence of the pathogen. Thus, treatment was discontinued in September 2006 and the patient remained well thereafter.

Discussion

The genus *Babesia* is phylogenetically related to other sporozoan genera, i.e. *Toxoplasma* and *Plasmodium*. To date, about 100 species of *Babesia* have been described world-wide based on intraerythrocytic stages detected in mammals (Levine, 1988). However, their pleomorphism in different species of mammalian hosts and the results of recent molecular systematic studies render some of these descriptions dubious (Gray, 2006; Hunfeld and Brade, 2004). It is now assumed that some species of *Babesia* are less host-specific than believed previously and that a number of species of *Babesia* will be reclassified as more information becomes available on them (Gray, 2006; Homer et al., 2000; Hunfeld and Brade, 2004). During the last decade, new *Babesia* spp. of unknown identity currently designated as WA1-type organisms (WA1-WA3 and CA1-CA6), MO1, and EU 1 have been established to be pathogens of considerable concern for humans in the USA and Europe (Herwaldt et al., 2003; Hunfeld and Brade, 2004; Conrad et al., 2006), with recent case reports suggesting that new species are emerging and that *Babesia* infections in European countries are more common than believed previously, especially in the immunocompromised host (Herwaldt et al., 2003; Hunfeld et al., 2002). Here, we describe the first reported human case of babsiosis in Germany and provide molecular evidence that the causative pathogen detected in our patient is virtually indistinguishable from the recently described *Babesia* organism designated as EU1 (Herwaldt et al., 2003). Molecular characterisation of the pathogen revealed that EU1 is not closely related to *Babesia* organisms such as *B. microti* or the recently established species *B. duncani* (formerly dubbed 'WA1' and 'CA5') that are known to have infected humans (Herwaldt et al., 2003; Conrad et al., 2006). Upon phylogenetic analysis, the newly discovered pathogen EU 1 instead clusters with *B. odocoilei*, a parasite of white-tailed deer, and these two organisms form a sister group with *B. divergens* (Herwaldt et al., 2003). Provisionally, the species name "*Babesia venatorum*" has been proposed for EU1 organisms (Herwaldt et al., 2003).

In the past, human babesiosis has been thought to occur rarely in Europe, with only about 30 reported cases and all but four clinical cases have been attributed to *B. divergens*, which has been diagnosed mainly in splenectomised patients in former Yugoslavia, France, and the British Isles (Gorenflot et al., 1998; Gray 2006; Homer et al., 2000). However, microbiological diagnosis of human babesiosis in Europe, so far, has been based mainly on the detection of the parasites in Giemsa-stained blood smears of patients with clinical symptoms of disease. Although helpful for diagnostic purposes, detection of parasites in blood smears by microscopy alone without additional molecular analysis of the pathogen does not provide reliable species identification. Therefore, it remains to be clarified whether many cases of human babesiosis in Europe that would have been attributed to *B. divergens* in the past based on traditional methods, in reality, are caused by other *Babesia* organisms (Gray, 2006; Herwaldt et al., 2003). In Europe, patients with *B. divergens* infection mainly presented with acute febrile haemolytic disease, and in the initial reports their clinical courses were almost always fatal. In contrast to the findings in human *B. divergens* infection, the first two cases of EU1 babesiosis in Italy and Austria, which occurred in two asplenic men with Hodgkin's disease and large B-cell lymphoma, respectively, were different in that the disease manifestation ranged from mild to moderately severe and both patients were cured after successful chemotherapy with clindamycin and/or quinine (Herwaldt et al., 2003). Whether this observation is typical of the clinical course of EU1 babesiosis, or whether differences in the pathogenicity of strains endemic to different geographic areas may explain the variable clinical disease manifestations, remains to be determined.

Similar to the manifestation in our patient clinical symptoms of the disease in the Italian and Austrian patients included elevated body temperature, chills, anaemia, weakness, fatigue, anorexia, and headache followed by jaundice and dark urine from haemoglobinuria. As with our patient, there was a significant delay of several days between the onset of symptoms, the detection of parasites in blood smears suggesting babesiosis, and the initiation of chemotherapy. Such a delay of treatment can be fatal because human babesiosis can be further complicated by central nervous system involvement, or severe complications, such as congestive heart failure, intravascular coagulation, renal failure, and respiratory distress syndrome if left untreated (Homer et al., 2000; Mylonakis, 2001). Patients with babesiosis, most of whom are immunocompromised or asplenic, therefore, should be regarded as medical emergencies and require immediate treatment to arrest haemolysis and prevent renal failure (Gelfand, 2000; Homer et al., 2000). Our case, however, is unique in that the patient remained seronegative for specific

antibodies for several months and suffered from relapse after the conclusion of initial treatment. Moreover, re-treatment with atovaquone and azithromyin for 2 months was unsuccessful in clearing the parasite and low-level parasitaemia persisted for several months despite maintenance therapy with atovaquone, possibly due to the previous combined application of rituximab and prednisolone, which have highly immunosuppressive effects. It is well known that treatment with corticosteroids and depressed cellular immunity are associated with severe clinical illness in human babesiosis (Telford and Maguire, 2005; Meldrum et al., 1992; Rosner et al., 1984). Humoral immunity, however, appears to be less important than cellular immunity in controlling the disease as demonstrated for example in a mouse model of *B. microti* infection because B-cell-deficient mice remain less susceptible to infection, whereas T-cell receptor-deficient mice are readily infected (Clawson et al., 2002). Whether such data hold true also for human infection with EU1 organisms remains to be demonstrated in future experiments. It is interesting to note though, that following prednisolone treatment and rituximab-induced depletion of CD20-positive cells, it took our patient 7 months to finally develop a *Babesia*-specific antibody response. At the same time, he obviously started clearing the parasite from his blood stream and smears turned negative upon microscopic examination. Six weeks later, PCR which had been used to monitor the success of treatment turned negative also (Table 1). The question whether seroconversion in our patient was simply a sign of immune reconstitution or whether the production of anti-*Babesia* antibodies significantly contributed to clearing the parasite, however, cannot be answered from our data.

So far, Giemsa-stained blood smears (Fig. 2a–c) remain the mainstay for the detection of parasites in patients with clinical symptoms of babesiosis. In addition, indirect detection of the pathogen can be performed by IFA for IgG and IgM antibodies. Obviously, in our patient, antibodies directed against the infecting parasite predominantly cross-reacted with *B. divergens* antigen in the IFA. This phenomenon was initially reported also by Herwaldt et al. (2003) in previous cases of EU 1 infection and suggests that assays using *B. divergens* antigen preparations can be helpful in the laboratory diagnosis of antibodies in such patients. One drawback of serology, however, is the fact that the production of antibodies in the non-immunocompromised host can take up to 14 days after the infection, and immunocompromised individuals can remain seronegative for even longer periods after the onset of clinical symptoms (Homer et al., 2000).

PCR is a new, rapid diagnostic technique for the detection of *Babesia* spp. that has not yet been standardised and systematically evaluated (Homer et al., 2000; Hunfeld and Brade, 2004). As with our case, however, diagnostic results with this method are promising and show PCR to be a sensitive and specific tool in the diagnosis of human babesiosis (Armstrong et al., 1998; Homer et al., 2000; Persing et al., 1992). Whether PCR indeed can be useful in monitoring the success of specific treatment in patients with prolonged persistent parasitaemia awaits further evidence from clinical studies.

Therapy of human babesiosis with a combination of quinine and clindamycin for 7–10 days is the most commonly used treatment. A recent prospective, randomised study in humans compared the efficacy of this treatment regimen with the clinical outcome of atovaquone and azithromycin in cases of *B. microti* infection. Although the clinical outcome was similar for the two patient groups, the application of atovaquone plus azithromycin lowered the relative number of side effects to 15% versus 72% in the group of patients receiving quinine and clindamycin (Krause et al., 2000). In our case, the application of atovaquone and azithromycin as re-treatment and maintenance therapy was well tolerated and proved effective in treating the clinical symptoms of the patients although it did not immediately clear the parasite from the blood stream. We did not use exchange transfusion in addition to conventional chemotherapy because this treatment is reserved for those individuals who are extremely ill, i.e., those with massive haemolysis, asplenia, immunosuppression, and blood parasitaemia of more than 10% (Mylonakis, 2001).

In Europe, *Babesia* spp. such as *B. microti*, *B. divergens*, *B. odocoilei*-like, and the newly described EU1 *Babesia* organism are known to be prevalent in *I. ricinus* (Duh et al., 2005; Hartelt et al., 2004). In Germany, *I. ricinus* is widely distributed and is regarded as the chief vector of tick-borne infections to humans (Hunfeld and Brade, 2004). *B. microti* has been identified in rodents, *B. divergens* in cattle, and both species in *I. ricinus* ticks in Germany (Hartelt et al., 2004; Huwer et al., 1994; Walter, 1984). Moreover, the potential relevance of *Babesia* species for tick-exposed German individuals was substantiated very recently by demonstrating the presence of *B. microti* or *B. divergens* in 31 (1%) out of 3113 investigated *I. ricinus* ticks collected in south western Germany by PCR and subsequent nucleotide sequence analysis of the small subunit ribosomal RNA gene (Hartelt et al., 2004). Few systematic studies have been conducted on potentially infected humans as yet, but recent seroepidemiological investigations using *B. divergens* and *B. microti* as surrogate antigens suggest that infections with *Babesia* spp. may occur more frequently than previously believed in tick-exposed patients in Europe, with overall seroprevalence rates ranging between 3.6% and 5.4% in the population investigated (Hunfeld et al., 2002). However, no such data are currently available for potentially emerging *Babesia* organisms such as EU1. When dealing with the actual frequency of *Babesia* infections in

European countries, it is also likely that in most immunocompetent individuals suffering from babesiosis the disease probably is self-limiting and milder than in the immunocompromised host (Hunfeld and Brade, 2004). The apparent existence and high prevalence of babesial pathogens in ticks, however, may become increasingly important as the number of immunocompromised individuals and organ transplant recipients is increasing steadily and because, although a rarity, asymptomatic but chronically infected blood donors are now known to be a source of transfusion-transmitted babesiosis in areas where *Babesia* spp. with zoonotic potential are endemic (Homer et al., 2000; Popovsky, 1991). Consequently, further seroepidemiological and molecular epidemiological studies on *Babesia* spp. in general and EU1-like organisms in particular are urgently needed to learn more about the true distribution and medical relevance of such pathogens in the various parts of Europe.

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研究報告の概要

○広範囲薬剤耐性結核(超薬剤耐性結核)の世界的新興

薬剤耐性結核(MDR TB)の治療に使用される第二選択剤に耐性を増しつつある*Mycobacterium tuberculosis*株は、世界各国の公衆衛生の脅威となりつつある。我々は、2000年～2004年の期間中に第二選択抗結核剤に耐性を示した*M. tuberculosis*分離菌株について、国際的に認知された結核研究施設(Supranational Reference Laboratories;SRL)のネットワークを調査した。広範囲薬剤耐性結核(XDR TB)は、第二選択剤6クラスのうち3つ以上に耐性を持つMDR TBと定義した。該当施設23のうち、14(61%)施設から分離菌株17,690のデータが提供され、これは48カ国の薬剤感受性結果を反映するものであった。MDR TB 分離菌株3,520(19.9%)のうち、347(9.9%)がXDR TBの基準を満たした。公衆衛生を守り、結核を制圧するには、集団での傾向をさらに調査し、薬剤耐性を防ぎMDR TB患者を効果的に治療するための努力を拡大することが極めて重要である。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2000年～2004年の期間中に第二選択抗結核剤に耐性を示した*M. tuberculosis*分離菌株について、国際的に認知された結核研究施設のネットワークを調査したところ、該当施設23のうち、14(61%)施設から分離菌株17,690のデータが提供され、347(9.9%)が広範囲薬剤耐性結核菌だったとの報告である。	日本赤十字社は、問診で結核の既往、薬剤の使用を確認し、既往がある場合は治癒するまで献血不可としている。また、体調不良者を献血不可としている。今後も引き続き情報の収集に努める。

RESEARCH

Worldwide Emergence of Extensively Drug-resistant Tuberculosis

N. Sarita Shah,*[1] Abigail Wright,† Gill-Han Bai,‡[2] Lucia Barrera,§[2] Fadila Boulahbal,¶[2] Nuria Martín-Casabona,#[2] Francis Drobniewski,**[2] Chris Gilpin,††[2] Marta Havelková,‡‡[2] Rosario Lepe,§§[2] Richard Lumb,¶¶[2] Beverly Metchock,*[2] Françoise Portaels,##[2] Maria Filomena Rodrigues,***[2] Sabine Rüsch-Gerdes,†††[2] Armand Van Deun,##[2] Veronique Vincent,‡‡‡[2,3] Kayla Laserson,* Charles Wells,* and J. Peter Cegielski*

Mycobacterium tuberculosis strains that are resistant to an increasing number of second-line drugs used to treat multidrug-resistant tuberculosis (MDR TB) are becoming a threat to public health worldwide. We surveyed the Network of Supranational Reference Laboratories for *M. tuberculosis* isolates that were resistant to second-line anti-TB drugs during 2000–2004. We defined extensively drug-resistant TB (XDR TB) as MDR TB with further resistance to ≥3 of the 6 classes of second-line drugs. Of 23 eligible laboratories, 14 (61%) contributed data on 17,690 isolates, which reflected drug susceptibility results from 48 countries. Of 3,520 (19.9%) MDR TB isolates, 347 (9.9%) met criteria for XDR TB. Further investigation of population-based trends and expanded efforts to prevent drug resistance and effectively treat patients with MDR TB are crucial for protection of public health and control of TB.

Multidrug-resistant tuberculosis (MDR TB) has been documented in nearly 90 countries and regions worldwide (*1*); 424,203 cases of MDR TB were estimated to have occurred in 2004, which is 4.3% of all new and previously treated TB cases (*2*). Treatment for MDR TB patients requires use of second-line drugs for ≥24 months. These drugs are more costly, toxic, and less effective than first-line drugs used for routine treatment of TB (*3–6*). As with other diseases, resistance to TB drugs results primarily from nonadherence by patients, incorrect drug prescribing by providers, poor quality drugs, or erratic supply of drugs (*7*).

To facilitate treatment of MDR TB in resource-limited countries, where most TB cases occur (*1,2*), the World Health Organization (WHO) and its partners developed the Green Light Committee, which helps ensure proper use of second-line drugs, to prevent further drug resistance (*8*). Nonetheless, the Green Light Committee encountered numerous anecdotal reports of MDR TB cases with resistance to most second-line drugs. Once a strain has developed resistance to second-line drugs, these new TB strains are even more difficult to treat with existing drugs. Untreated or inadequately treated patients are at increased risk of spreading their disease in the community, which could lead to outbreaks in vulnerable populations and widespread emergence of a lethal, costly epidemic of drug-resistant TB, reminiscent of the MDR TB outbreaks in the early 1990s (*9–13*). Therefore, to determine whether these anecdotal reports were isolated events, early evidence of an emerging epidemic, or the occurrence of virtually

*Centers for Disease Control and Prevention, Atlanta, Georgia, USA; †World Health Organization, Geneva, Switzerland; ‡Korean Institute of Tuberculosis, Seoul, Republic of Korea; §National Institute of Infectious Diseases, Buenos Aires, Argentina; ¶Institut Pasteur d'Algérie, Alger, Algeria; #Hospital Universitaris Vall d'Hebron, Barcelona, Spain; **Health Protection Agency, London, United Kingdom; ††Prince Charles Hospital, Brisbane, Queensland, Australia; ‡‡National Institute of Public Health, Scrobarova, Czech Republic; §§Institute of Public Health of Chile, Providencia Santiago, Santiago, Chile; ¶¶Institute of Medical and Veterinary Science, Adelaide, South Australia, Australia; ##Institute of Tropical Medicine, Antwerp, Belgium; ***National Institute of Health, Porto, Portugal; †††National Reference Center for Mycobacteria, Borstel, Germany; and ‡‡‡Institut Pasteur, Paris, France

[1]Current affiliation: Albert Einstein College of Medicine, Bronx, New York, USA

[2]Member: World Health Organization/International Union against Tuberculosis and Lung Diseases Network of Supranational Reference Laboratories

[3]Current affiliation: World Health Organization, Geneva, Switzerland

untreatable forms of drug-resistant TB that had not been described previously in different parts of the world, we characterized and quantified the frequency of second-line–drug resistance in several geographic regions.

We sought to determine the extent to which highly resistant *Mycobacterium tuberculosis* strains have been identified by the international laboratories that participate in the Network of Supranational Reference Laboratories (SRLs). The SRL Network consists of 25 highly proficient TB laboratories on 6 continents. These laboratories collaborate with national reference laboratories to strengthen culture and drug-susceptibility testing capacity and to provide quality control for the WHO/International Union Against Tuberculosis and Lung Diseases Global Project on Anti-TB Drug Resistance (*14*).

Methods

Participants

From November 2004 through November 2005, we surveyed the global SRL Network. All SRL directors were invited to participate during the 2004 annual SRL directors meeting, by individual mailings, and by personal phone calls. Drug-susceptibility testing results were requested for *M. tuberculosis* isolates that had been tested for resistance to first-line drugs and second-line drugs during 2000–2004. Two SRLs were not eligible because they did not test for second-line drugs or tested for <3 classes of second-line drugs.

The 14 SRLs that provided data for this study support 112 TB laboratories in 80 countries worldwide (Figure 1). SRLs serve as international reference laboratories to a wide geographic area, performing drug-susceptibility testing that may not be available in a country (e.g., for second-line drugs) and providing quality assurance for first-line–drug testing. Most SRLs also serve as the national reference laboratory for the country in which they are located; they receive varying proportions of isolates from their own and other countries for surveillance, clinical diagnosis, and quality assurance. First-line–drug susceptibility testing is performed on all isolates; second-line–drug susceptibility testing is usually limited to isolates from patients known or suspected to have drug-resistant TB. Of the 14 participating SRLs, not all tested for all 6 classes of second-line drugs, and 4 did not submit data for the entire survey period.

In contrast, the SRL in the Republic of Korea serves as the national reference laboratory and routinely performs an extended diagnostic panel of drug-susceptibility testing on isolates from culture-positive TB patients referred from health centers, hospitals, and clinics in the Republic of Korea. This SRL tests all isolates for 6 classes of second-line drugs; thus, data from the Republic of Korea reflect most culture-positive cases and provide a close approximation to a population estimate of prevalence. Because of the large number of isolates received and because sampling for these isolates is systematically different from that at the other SRLs (testing of all TB patients in the Republic of Korea vs testing of patients more likely to have drug-resistant TB in other SRLs), resistance patterns for the Republic of Korea were analyzed separately from those for the other SRLs.

Laboratory Methods

Among participating SRLs, different but internationally accepted methods were used to test for second-line drug resistance (details available upon request). Validation of drug-susceptibility testing results for second-line drugs was not performed as part of this survey, but as part of their role as global reference laboratories, all SRLs participate in international proficiency testing for first-line drugs. Quality assurance procedures for second-line–drug susceptibility testing have not been developed; as a proxy for quality assurance, we examined the accuracy of second-line–drug susceptibility testing among isolates susceptible to the 4 main first-line drugs (isoniazid [INH], rifampin [RIF], ethambutol, and streptomycin). On the basis of known mechanisms of drug resistance, finding an isolate that is susceptible to all first-line drugs and resistant to second-line drugs is unlikely (*7*).

Procedures and Definitions

A standardized reporting form requested anonymous data for all isolates tested for resistance to ≥3 second-line drug classes during 2000–2004. Data were abstracted from the records, electronic or paper, depending on laboratory practices for data management. Results were submitted for 1 isolate per patient. Because SRLs rarely receive multiple isolates from the same patient, reporting of the same patient more than once was unlikely (B. Metchock and

Figure 1. Shading indicates 48 countries that submitted at least 1 isolate to participating Supranational Reference Laboratories, 2000–2004. See Table 4 for complete list of participating countries.

G.H. Bai, pers. comm.). No specimens were collected for this study; we used only data from records of isolates that had already been tested. Limited clinical information about the patient was available with each isolate. Consistent data were available for country of origin and date of drug-susceptibility testing. Data about age and TB treatment history were available for <10% of patients, so analysis was not considered reliable for these variables.

To best compare data for the study samples with data from the Global Drug Resistance Survey and other population-based drug-resistance surveillance, we analyzed first-line–drug resistance patterns according to standard methods used in anti-TB–drug resistance surveys (*1*). These patterns included any drug resistance, monoresistance (resistance to only the 1 specified drug), polyresistance (resistance to ≥2 first-line drugs, but which drugs not specified), and multidrug resistance (resistance to at least INH and RIF, with or without other drugs).

We defined 6 classes of second-line drugs as follows: aminoglycosides other than streptomycin (e.g., kanamycin and amikacin), cyclic polypeptides (e.g., capreomycin), fluoroquinolones (e.g., ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin), thioamides (e.g., prothionamide and ethionamide), serine analogs (e.g., cycloserine and terizidone), and salicylic acid derivatives (e.g., para-aminosalicyclic acid).

For this survey we created a consensus definition that incorporates second-line–drug susceptibility results and is based on international guidelines for management of drug-resistant TB (*15*). The mainstay of an MDR TB treatment regimen consists of 1 injectible drug (e.g., aminoglycoside or cyclic polypeptide) and a fluoroquinolone; additional drugs from the remaining classes are added until the total reaches 4–6 drugs to which the organism is susceptible. If the infecting organism is resistant to ≥3 second-line drug classes, designing a treatment regimen with sufficient drugs that are known to be effective against TB is difficult. Thus, we defined extensively drug-resistant TB (XDR TB) isolates as those meeting the criteria established for MDR TB plus resistance to ≥3 of the 6 classes of second-line drugs.

Second-line–drug resistance patterns were analyzed by geographic region from which the isolate was submitted to the SRL. Regions were grouped into epidemiologically meaningful categories on the basis of prevalence of TB and MDR TB (*1,16*). This retrospective survey was evaluated and approved as public health surveillance by the US Centers for Disease Control and Prevention (CDC).

Results

We received data for 18,462 patients from 14 (61%) of 23 eligible SRLs. We excluded those patients tested before 2000 (n = 223), tested after 2004 (n = 14), or tested for resistance to <3 classes of second-line drugs (n = 535). Our final study sample consisted of 17,690 patients whose isolates were tested for resistance to ≥3 second-line drugs during 2000–2004 (Figure 2). Of these, 11,939 (67.5%) patients were from the Republic of Korea and 5,751 (32.5%) were from the remaining SRLs.

First-line–Drug Susceptibility

Among isolates from patients from the 13 SRLs other than the Republic of Korea, 3,765 (65.5%) were resistant to ≥1 first-line TB drug (Table 1). Of these, 3,305 (58.5%) were resistant to at least INH and 2,345 (41.5%) were resistant to at least RIF. Among isolates from the Republic of Korea patients, 2,508 (21%) had resistance to any drug; most (n = 2,196; 18.4%) were resistant to INH.

Single-drug resistance was found for isolates from 884 (15.4%) patients from the 13 SRLs; 456 (8.1%) of these were resistant to INH and 99 (1.8%) to RIF. Among isolates from patients from the Republic of Korea, 952 (8%) displayed single-drug resistance, 666 (5.6%) to INH and 148 (1.2%) to RIF.

Polyresistance other than MDR TB was seen for isolates from 651 (11.5%) patients from the 13 SRLs and 258 (2.2%) from the Republic of Korea SRL. Not all SRLs routinely tested for resistance to pyrazinamide.

Multidrug resistance (i.e., MDR TB) was present in isolates from 2,222 (39.4%) patients from the 13 SRLs and 1,298 (10.9%) from the Republic of Korea. Resistance to

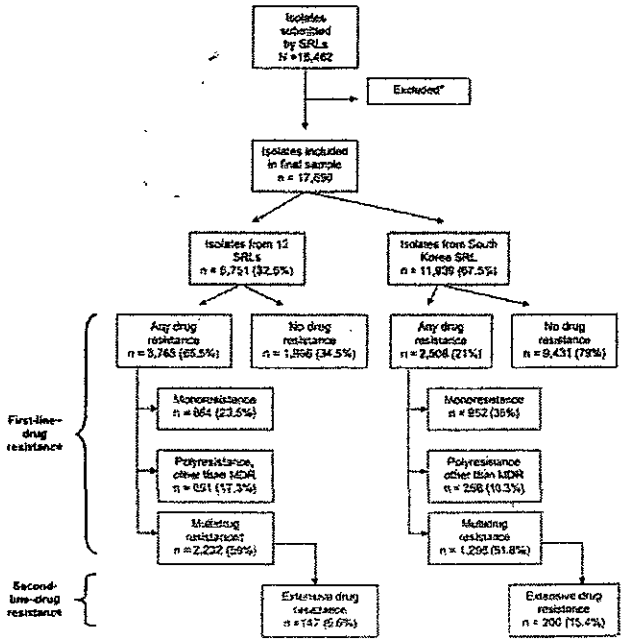

Figure 2. Selection of study sample and summary of drug-resistance patterns of isolates. SRL, Supranational Reference Laboratory. *Tested before 2000 or after 2004 (n = 247) or tested for resistance to <3 classes of second-line drugs (n = 535). †Data for ethambutol resistance missing for 5 isolates.

Table 1. First-line–drug resistance patterns for *Mycobacterium tuberculosis* isolates, 2000–2004 (N = 17,690)*

Pattern	Other 13 SRLs (n = 5,751)		Republic of Korea SRL (n = 11,939)	
	No. tested	No. (%) resistant	No. tested	No. (%) resistant
Any resistance (total)*†‡	5,751	3,765 (65.5)	11,939	2,508 (21.0)
INH	5,645	3,305 (58.5)	11,939	2,196 (18.4)
RIF	5,649	2,345 (41.5)	11,939	1,469 (12.3)
EMB	5,508	1,356 (24.6)	11,939	988 (8.3)
SM	5,618	2,581 (45.9)	11,939	578 (4.8)
Monoresistance (total)§¶	5,751	884 (15.4)	11,939	952 (8.0)
INH	5,645	456 (8.1)	11,939	666 (5.6)
RIF	5,649	99 (1.8)	11,939	148 (1.2)
EMB	5,508	8 (0.1)	11,939	25 (0.2)
SM	5,618	321 (5.7)	11,939	113 (0.9)
Polyresistance, non-MDR (total)¶	5,644	651 (11.5)	11,939	258 (2.2)
INH + other drugs (except RIF)	5,645	627 (11.1)	11,939	232 (1.9)
RIF + other drugs (except INH)	5,649	24 (0.4)	11,939	23 (0.2)
Multidrug resistance (total)¶#	5,644	2,222 (39.4)	11,939	1,298 (10.9)
INH + RIF, only	5,644**	399 (7.1)	11,939	392(3.3)
INH + RIF + EMB, only	5,508**	182 (3.3)	11,939	584 (4.9)
INH + RIF + SM, only	5,618**	619 (11.0)	11,939	89 (0.7)
INH + RIF + EMB + SM	5,476**	1,017 (18.6)	11,939	233 (2.0)

*SRLs, Supranational Reference Laboratories; INH, isoniazid; RIF, rifampin; EMB, ethambutol; SM, streptomycin.
†Missing data for INH (106 isolates), RIF (102 isolates), EMB (243 isolates), SM (133 isolates).
‡Cells are not mutually exclusive.
§Numerator is isolates with resistance to the specified drug and no known resistance to other first-line drugs. Denominator is isolates tested to at least the specified drug in the numerator.
¶Each cell is mutually exclusive.
#Denominator is isolates tested for at least INH + RIF.
**Denominator is isolates tested for at least the drugs in the specified combination.

all first-line drugs tested (i.e., MDR TB with additional resistance to ethambutol and streptomycin) was found in isolates from 1,017 (18.6%) patients from the 13 SRLs and 233 (2%) from the Republic of Korea SRL.

Second-line–Drug Susceptibility

Among patients from the 13 SRLs, resistance to aminoglycosides was detected in 489 (8.7%) isolates and to fluoroquinolones in 298 (5.3%) (Table 2). Among isolates from Republic of Korea patients, resistance was most commonly seen to fluoroquinolones (n = 524, 4.4%) and thioamides (n = 259, 2.2%).

From all SRLs, isolates that were resistant to at least INH and RIF (i.e., MDR TB; n = 3,520) and tested for susceptibility to ≥3 second-line drugs were combined for analysis of second-line–drug resistance patterns. Resistance to ≥1 class of second-line drug was present in 1,542 (43.8%) MDR TB patients (Table 3). The most commonly observed patterns were resistance to aminoglycosides (n = 630, 18.3%), fluoroquinolones (n = 673, 19.3%), and thioamides (n = 605, 19.3%).

MDR TB patients whose isolates had further resistance to ≥3 classes of second-line drugs were classified as XDR TB (Table 3). A total of 347 (9.9%) MDR TB patients met criteria for XDRTB. According to the revised Global XDR TB Task Force definition (www.who.int/mediacentre/news/notes/2006/np29/en/index.html), 234 (6.6%) isolates met criteria for XDR TB. Among XDR TB patients, combination drug-resistance patterns included 90 (3.4%) with resistance to aminoglycosides, capreomycin

Table 2. Second-line–drug resistance patterns for *Mycobacterium tuberculosis* isolates, 2000–2004 (N = 17,690)*†

Pattern	Other 13 SRLs‡ (n = 5,751)		Republic of Korea SRL‡ (n = 11,939)	
	No. tested	No. (%) resistant	No. tested	No. (%) resistant
Any resistance	5,751	1,237 (21.5)	11,939	849 (7.1)
Aminoglycosides§	5,620	489 (8.7)	11,939	227 (1.9)
Capreomycin	4,347	197 (4.5)	11,939	122 (1.0)
Fluoroquinolones	5,580	298 (5.3)	11,939	524 (4.4)
Thioamides	5,131	556 (10.8)	11,939	259 (2.2)
Cycloserine	2,715	70 (2.6)	11,939	80 (0.7)
Para-aminosalicylic acid	3,571	262 (7.3)	11,939	403 (3.4)

*SRLs, Supranational Reference Laboratories.
†Not all isolates were tested for each second-line–drug class (with the exception of the Republic of Korea SRL), so results are reported as a proportion of isolates tested to the specified class of drugs.
‡Cells are not mutually exclusive.
§Other than streptomycin (e.g., kanamycin, amikacin).

Table 3. Second-line–drug resistance patterns for multidrug-resistant *Mycobacterium tuberculosis* isolates, 2000–2004*†‡

Pattern	No. tested	No. (%) resistant
Any resistance (total)	3,520	1,542 (43.8)
Aminoglycosides (AG)§	3,442	630 (18.3)
Capreomycin (CM)	2,743	279 (10.2)
Fluoroquinolones (FQ)	3,492	673 (19.3)
Thioamides (TA)	3,132	605 (19.3)
Cycloserine (CS)	2,615	141 (5.4)
Para-aminosalicylic acid (PAS)	2,860	450 (15.7)
Extensively drug-resistant TB (XDR TB, total)¶	3,520	347 (9.9)
AG + CM + FQ	2,656	90 (3.4)
AG + CM + TA	2,498	77 (3.1)
CM + FQ + TA	260	50 (19.2)
AG + FQ + TA	3,040	102 (3.4)
AG + FQ + CS	139	39 (28.1)
FQ + TA + PAS	2,505	94 (3.8)

*Tested for ≥3 second-line drug classes; SRLs, Supranational Reference Laboratories.
†Not all isolates were tested for each second-line drug class (with the exception of the Republic of Korea SRL), so results are reported as a proportion of isolates tested to the specified class of drugs. For combination resistance patterns, results are reported as a proportion of isolates tested to all of the classes of drugs in the specific combination.
‡Cells are not mutually exclusive.
§Other than streptomycin (e.g., kanamycin, amikacin).
¶XDR TB, extensively drug-resistant tuberculosis, i.e., multidrug-resistant tuberculosis (resistant to at least isoniazid and rifampin) with additional resistance to ≥3 classes of second-line drugs.

and fluoroquinolones; 102 (3.4%) with resistance to aminoglycosides, fluoroquinolones, and thioamides; and 94 (3.8%) with resistance to fluoroquinolones, thioamides, and para-aminosalicyclic acid. Nearly half (n = 167, 48.1%) of all XDR TB isolates were resistant to all 4 first-line drugs, bringing the total to ≥7 drugs to which the isolate was resistant.

The proportion of XDR TB patients by region is shown in Table 4. Among the group of industrialized nations, 53 (6.5%) MDR TB patients met criteria for XDR TB. Among patients from Russia and Eastern Europe, 55 (13.6%) MDR TB patients met criteria for XDR TB. Among patients from the Republic of Korea, 200 (15.4%) MDR TB patients, who accounted for 1.7% of all *M. tuberculosis* isolates tested, met criteria for XDR TB.

In evaluating the accuracy of second-line–drug susceptibility testing, we found that 7 (0.1%) of 11,426 patients fully susceptible to all first-line drugs were resistant to 2 second-line drugs, and 109 (1%) were resistant to 1 second-line drug. Most of these patients were resistant to fluoroquinolones.

Discussion

This study represents the first assessment of the widespread occurrence of *M. tuberculosis* with such extensive drug resistance as to be nearly untreatable with currently available drugs, according to international guidelines. We provide data on second-line–drug resistance for the largest sample of patients to date, including >5,000 patients from 47 countries, apart from the Republic of Korea. The definition of XDR TB in this survey is based on WHO guidelines for the programmatic management of drug-resistant TB; the guidelines recommend treatment with ≥4 drugs known to be effective (*15*). Therefore, with ≤3 remaining classes of second-line drugs to which the infecting organism is susceptible, treatment of these patients cannot meet international standards. XDR TB has been detected in all regions of the world. XDR TB strains in this study also have high rates of resistance to pyrazinamide and ethambutol, thereby severely limiting the treatment options available.

Analysis of combination second-line–drug resistance patterns is critical for clinicians and policymakers who design treatment regimens for these patients. Although limited data exist in the literature about second-line–drug resistance patterns among MDR TB patients, data from patients undergoing retreatment for TB in Hong Kong showed that 30 (17%) MDR TB isolates were resistant to ≥3 second-line drugs (*17*), thereby meeting criteria for XDR TB. A drug-resistance survey of 447 culture-positive new patients and patients undergoing retreatment in Abkhazia, Republic of Georgia, found that of 63 MDR TB patients, 2 (3%) had additional resistance to 3 second-line drug classes, consistent with XDR TB (*18*). More recently, clusters of XDR TB have been reported in South Africa and Iran (*19,20*) and have been associated with HIV infection and rapid and high death rates.

The emergence of new strains of TB that are resistant to second-line drugs, especially in settings where TB control programs have become unable to adequately monitor treatment regimens for MDR TB, is cause for concern. After the resurgence of TB in industrialized countries during the 1980s and increased awareness of this global problem, implementation of strong TB control programs based on the principles of the global directly observed treatment strategy, short course (DOTS) improved treatment outcomes and reduced TB and MDR TB incidence in several countries. This framework for DOTS, promulgated by WHO, and the pilot MDR TB management projects (DOTS-Plus projects) became the basis for programmatic management of MDR TB, which has demonstrated feasibility and effectiveness in low- and middle-income countries (*5,15*). However, second-line drugs are available worldwide outside of well-organized TB-control programs (WHO, unpub. data).

Improper treatment of drug-resistant TB, such as using too few drugs, relying on poor quality second-line drugs, and failing to ensure adherence to treatment, will likely lead to increases in XDR TB. Strengthening basic TB programs and infection control measures is crucial for preventing the selective pressure and environments in which resistant strains are transmitted from person to per-

Table 4. Extensively drug-resistant tuberculosis among multidrug-resistant tuberculosis isolates, by region, 2000–2004*

Geographic region	Total no. isolates tested, n†	Total MDR TB patients, n (% of all isolates tested)	Total XDR TB patients, n (% of MDRTB patients)
Industrialized nations‡	2,499	821 (32.9)	53 (6.5)
Latin America§	985	543 (55.1)	32 (5.9)
Eastern Europe¶ and Russia	1,153	406 (35.2)	55 (13.6)
Africa and Middle East#	665	156 (23.5)	1 (0.6)
Asia (other than Republic of Korea)**	391	274 (70.1)	4 (1.5)
Republic of Korea	11,939	1,298 (10.9)	200 (15.4)
Total††		3,418	345

*Region from which isolate was submitted to Supranational Reference Laboratory. MDR TB, multidrug-resistant tuberculosis; XDR TB, extensively drug-resistant tuberculosis, i.e., multidrug-resistant tuberculosis (resistant to at least isoniazid and rifampin) with additional resistance to ≥3 classes of second-line drugs.
†Total no. of isolates tested for resistance to ≥3 second-line drug classes, including aminoglycosides (amikacin or kanamycin), polypeptides (capreomycin), fluoroquinolones (ofloxacin or ciprofloxacin), thioamides (ethionamide or prothionamide), cycloserine, and para-aminosalicyclic acid.
‡United States, Canada, United Kingdom, countries in Western Europe (Ireland, Portugal, Germany, France, Belgium, Spain), Japan, and Australia.
§Argentina, Bolivia, Brazil, Chile, Ecuador, Guyana, French Guiana, Peru, Mexico, Guatemala, El Salvador, Costa Rica.
¶Republic of Georgia, Czech Republic, Azerbaijan, Armenia.
#Afghanistan, Algeria, Egypt, Tunisia, Botswana, Burundi, Cameroon, Central African Republic, Côte d'Ivoire, Djibouti, Madagascar, Rwanda, South Africa, Senegal, Uganda.
**Bangladesh, Indonesia, Papua New Guinea, Thailand, East Timor.
††For 2 XDR TB patients, data were missing about geographic region.

son. Additionally, MDR TB programs that rely on quality-assured and internationally recommended treatment regimens according to WHO guidelines must be scaled up and strengthened to stem further second-line–drug resistance and spread of XDR TB. The Green Light Committee provides a global mechanism to help affected countries achieve these steps. A commentary published in 2000 predicted that "failure to institute [the] entire DOTS-Plus package is likely to destroy the last tools available to combat [TB], and may ultimately result in the victory of the tubercle bacillus over mankind" (*21*). XDR TB is an indirect indicator of program failure to adequately diagnose, prevent, and treat MDR TB.

Documenting the emergence of XDR TB requires a laboratory-based diagnosis that relies on first- and second-line–drug susceptibility testing. A limitation to accurate detection of XDR TB is that existing tests for resistance to second-line drugs are not yet standardized and are less reproducible than tests for resistance to INH and RIF. Lack of international recommendations for use, as well as lack of standardization and the historical unavailability of MDR TB treatment in the public sector, has limited use of second-line–drug susceptibility testing on a wider scale. As access to treatment with second-line drugs increases, standardized methods, improved diagnostics, and quality assurance for second-line–drug susceptibility testing are urgently needed to enable reliable testing and design of appropriate treatment regimens. Although internationally accepted methods were used by all laboratories, the precise methods and drug concentrations used varied among participating SRLs (*22*). Because these SRLs represent some of the most highly performing laboratories on 6 continents, results of drug-susceptibility testing are credible within the context of stated limitations. Initial studies that standardized different methods for second-line–drug susceptibility testing have been completed (*23–26*), but more are needed.

Our study has other limitations. The numbers reported for XDR TB probably represent an underestimate of the true number of cases because not all SRLs and not all national reference laboratories test for all 6 classes of second-line drugs. In the absence of test results for all 6 classes of second-line drugs, we speculate, on the basis of a patient's TB treatment history and known patterns of drug cross-resistance, that many other unidentified patients are likely to have had and died from XDR TB. For example, an MDR TB isolate that is also resistant to an aminoglycoside and a fluoroquinolone but that has not been tested for the other second-line drug classes is very likely to be resistant to an additional second-line drug class for the following reasons: INH and ethionamide have a 15%–20% rate of cross-resistance (*27*); kanamycin and capreomycin cross-resistance is common, ranging from 20%–60% (CDC, unpub. data) (*28,29*); and in this study, isolates that were resistant to all 4 first-line drugs as well as an aminoglycoside and a fluoroquinolone were 70%–80% likely to be resistant to at least 1 additional class of second-line drug.

Another limitation is that data from most SRLs were drawn from a convenience sample of isolates and reflect referral bias. Thus, these data can not be considered representative of a patient population or region, and actual denominators are difficult to determine. For this reason, although estimates of prevalence are possible, they cannot be generalized to the local or regional population. However, our study is the first to report XDR TB patients in multiple geographic regions; future systematic surveys are needed to determine the true extent of this disease. Data from the Republic of Korea reflect a more comprehensive policy for drug-susceptibility testing and provide an estimate of the population prevalence in this setting.

However, the 10.9% rate of MDR TB for the Republic of Korea is higher than rates reported from other national drug resistance surveys and may reflect other unknown referral biases (*1*).

Lastly, we had limited clinical information about each patient because information submitted to each SRL varied and was not reliably available for inclusion in the analysis. Data about TB treatment history, patient age and sex, or HIV status are not routinely collected by all laboratories. Genotyping data were not available to confirm whether XDR TB isolates are related to W variant of the Beijing strain, a highly drug-resistant strain of *M. tuberculosis* responsible for large nosocomial outbreaks in New York in the early 1990s (*30*).

Despite these limitations, our survey provides the first documentation of the emergence of XDR TB as a serious worldwide public health threat. XDR TB was identified on 6 continents and is significantly associated with worse treatment outcomes than MDR TB (*31,32*). The emergence of XDR TB, coupled with the increased use of second-line drugs, suggests that urgent measures are needed to improve rational use of quality-assured second-line drugs. In addition, population-based surveillance for second-line–drug susceptibility testing is needed to better describe the magnitude of XDR TB worldwide, track trends, and plan a public health response. Indeed, the convergence of XDR TB with the HIV epidemic may undermine gains in HIV prevention and treatment programs and requires urgent interventions. These interventions include ensuring adherence to recommended international standards of care aimed at promptly and reliably diagnosing TB, ensuring adherence to recommended treatment regimens with demonstrated efficacy, implementing infection control precautions where patients congregate, and improving laboratories' capacity to accurately and rapidly detect drug-resistant *M. tuberculosis* isolates so that patients can receive effective treatment (*33*). Other unmet needs include further development of international standards for second-line–drug susceptibility testing, new anti-TB drug regimens, and better diagnostic tests for TB and MDR TB. Such measures are crucial if future generations are to be protected from potentially untreatable TB.

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Dr Shah is an internist and epidemiologist with Albert Einstein College of Medicine. She is also a guest researcher with the Division of Tuberculosis Elimination at CDC, where she was an Epidemic Intelligence Service Officer at the time of this study. Her research interests focus on TB and HIV coinfection, drug resistance, and global health.

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Address for correspondence: N. Sarita Shah, Department of Medicine, Division of General Internal Medicine, Albert Einstein College of Medicine, 111 E. 210 St, Bronx, NY 10467, USA; email: sshah@montefiore.org

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研究報告の概要

○アフェレーシス血小板の細菌スクリーニング及び輸血による敗血症の残存リスク:米国赤十字の経験(2004～2006年)

背景:米国赤十字は2004年3月にすべてのアフェレーシス血小板(PLT)の細菌検査を開始したが、それでもなお、検査済み製剤による輸血後敗血症の報告がある。

試験デザイン及び方法:前方視的品質管理テスト(QC)及び細菌検査陰性のアフェレーシスPLTにより生じた敗血症の報告の調査から確認された敗血症とアフェレーシスPLTの細菌汚染率を、採取方法ごとに解析した。

結果:2004年3月1日～2006年5月31日の期間に、1,004,206本の供血血液に細菌培養テストが行われた。そのうち186(1:5,399)本に陽性の培養結果が確認された。293製剤のうち1件を除くすべての輸血が回避された。両腕法を用いて採取した製剤では、片腕法と比較して、確認された陽性細菌培養の割合が有意に高かった(105供血当り22.7vs.11.9; オッズ比[OR], 1.9; 95%信頼区間[CI], 1.4-2.7)。この期間中に、死亡3名(死亡/供血数1:498,711供血)を含む20例の敗血症が報告され、これはスクリーニング結果陰性のアフェレーシスPLTに関係したものであった。両腕法(1:41,173; 95% CI, 1:25,000-1:66,667)を用いて採取した場合の敗血症性副作用の頻度は、片腕法(1:193,305; 95% CI, 1:52,632-1:500,000; OR, 4.7; 95% CI, 1.2-18.4)と比較して4.7倍高かった。ほとんどの敗血症性副作用(16/20)は、*Staphylococcus spp.*を起炎菌とし、採血後5日目(13/ 20)に発現した。

結論:当該試験期間中においてQC培養による検出をすり抜けたPLTの細菌汚染(特に古いPLTや、両腕法で採取された血液成分中の皮膚常在菌など)は、いまだに輸血の重大な残存リスクである。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2004年3月1日～2006年5月31日の期間に、細菌培養で陽性となったアフェレーシス血小板293製剤のうち1件を除くすべての輸血が回避されたが、スクリーニング陰性の製剤に関係した敗血症が20例(うち死亡3例)報告された。両腕法を用いて採取した場合の敗血症性副作用の頻度は、片腕法と比較して4.7倍高かったとの報告である。	日本赤十字社では、輸血情報リーフレット等により、細菌感染やウイルス感染について医療機関へ情報提供し注意喚起している。また、「血液製剤等に係る遡及調査ガイドライン」(平成17年3月10日付薬食発第0310009号)における「本ガイドライン対象以外の病原体の取扱い イ. 細菌」に準じ細菌感染が疑われる場合の対応を医療機関に周知する。 今後も細菌感染に関する情報の収集に努める。

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Bacterial screening of apheresis platelets and the residual risk of septic transfusion reactions: the American Red Cross experience (2004-2006)

Anne F. Eder, Jean M. Kennedy, Beth A. Dy, Edward P. Notari, John W. Weiss, Chyang T. Fang, Stephen Wagner, Roger Y. Dodd, Richard J. Benjamin, and the American Red Cross Regional Blood Centers

BACKGROUND: The American Red Cross initiated systemwide bacterial testing of all apheresis platelet (PLT) collections in March 2004, yet continues to receive reports of septic reactions after transfusion of screened components.

STUDY DESIGN AND METHODS: The rates of confirmed bacterial contamination of apheresis PLT collections detected by prospective quality control (QC) testing, and by surveillance of reported septic reactions to screened-negative apheresis PLTs, were analyzed according to the technology utilized for collection.

RESULTS: Between March 1, 2004, and May 31, 2006, bacterial culture testing was performed on 1,004,206 donations; of these, 186 (1:5,399) had confirmed-positive culture results. Transfusion of all but 1 of the associated 293 components was prevented. A significantly higher rate of confirmed-positive bacterial cultures was seen with products collected utilizing two-arm collection procedures compared to one-arm procedures (22.7 vs. 11.9 per 10^5 donations; odds ratio [OR], 1.9; 95% confidence interval [CI], 1.4-2.7). During this period, 20 septic transfusion reactions were reported, including 3 fatalities (1:498,711 fatalities per distributed component), which implicated screened-negative apheresis PLT products. The frequency of septic reactions was 4.7-fold higher for collections utilizing two-arm procedures (1:41,173; 95% CI, 1:25,000-1:66,667) compared to collections from one-arm procedures (1:193,305; 95% CI, 1:52,632-1:500,000; OR, 4.7; 95% CI, 1.2-18.4); most septic reactions (16 of 20) were due to *Staphylococcus* spp. and occurred on Day 5 (13 of 20) after collection.

CONCLUSION: PLT contamination with bacteria that evade detection by QC culture remains a significant residual transfusion risk, in particular for older PLTs and skin-commensal bacteria in components collected by two-arm apheresis procedures during the study period.

Bacterial contamination of platelet (PLT) components remains a significant infectious risk to transfusion recipients, despite the implementation of preventive measures.[1-3] Contamination occurs from the introduction of low concentrations of skin bacteria at the time of phlebotomy; less commonly, from asymptomatic donor bacteremia; or rarely, during blood processing.[4] The low initial bacterial inoculum may lead to gross contamination during the 5-day storage period allowed for PLTs.[5,6] Septic transfusion reactions to PLT transfusion were estimated to occur with approximately 1:25,000 (range, 1:13,000-1:100,000) transfusions before 2004.[7-9] Actual contamination rates were measured to be substantially higher at 1:2,000 to 1:3,000 transfused PLT products.[3,10] The difference may be explained by low-level contamination that did not evoke a clinical response or the difficulty in recognizing septic transfusion reactions in patients with neutropenia or concomitant fever or on antibiotic therapy.

In 2004, professional standards were introduced by AABB that required measures to limit and detect bacterial contamination in all PLT components.[11] In response, blood centers and transfusion services implemented a variety of different preventive measures and bacterial

From Biomedical Services, Medical Office, National Headquarters, American Red Cross, Washington, DC; the Jerome H. Holland Laboratory, Rockville, Maryland; and the Badger Hawkeye Region, American Red Cross, Madison, Wisconsin.

Address reprint requests to: Dr Anne Eder, MD, PhD, Biomedical Services, National Headquarters, American Red Cross, 2025 E Street NW, Washington, DC 20006; e-mail: EderA@usa.redcross.org.

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detection strategies. In March 2004, the American Red Cross began routine in-process quality control (QC) testing for bacterial contamination in all apheresis PLTs with an automated microbial detection system (BacT/ALERT 3D, bioMérieux, Durham, NC).[12,13] Despite these efforts, septic reactions and rare fatalities linked to bacterially contaminated apheresis PLTs still occur, suggesting a substantial rate of false-negative bacterial culture results. We performed a retrospective analysis of our QC bacterial testing results and clinical reports of septic transfusion reactions to identify risk factors for bacterial contamination and transfusion-associated sepsis associated with apheresis PLTs.

MATERIALS AND METHODS

Collection of apheresis PLTs

Between March 1, 2004, and May 31, 2006, apheresis PLTs were collected utilizing standard protocols, from volunteer donors at 35 regional blood centers of the American Red Cross with one of three apheresis devices (Amicus, Baxter Healthcare, Round Lake, IL; Spectra, Gambro BCT, Lakewood, CO; or Trima, Gambro BCT), as previously described.[12] The Amicus and the Spectra devices have two configurations for collection, a continuous-separation protocol that requires an intravenous (IV) line in both arms for blood withdrawal (inlet line) or venous return (two-arm procedure; Amicus collection set, 4R2310; Spectra collection set, 777-003-015) and a discontinuous protocol that intermittently withdraws and returns blood through a single IV access line (one-arm procedure; Amicus collection set, 4R2312; Spectra collection set, 777-003-215). The Trima device utilizes a discontinuous protocol with single IV line access (Trima collection sets 777800100 and 777800400). All collection sets divert the initial 40 to 50 mL of whole blood drawn into a secondary collection container or tubes for viral marker and immunohematology testing. For two-arm procedures performed on the Amicus and Spectra technologies included in this report, the sample was collected from the first line placed during operation, which was the venous return line, not the inlet draw line.

The phlebotomy site is prepared by a standard, FDA-recommended, skin disinfection protocol utilizing povidone-iodine scrubs (or chlorhexidine gluconate in 70% isopropyl alcohol scrubs in donors allergic to iodine) in all Red Cross regional blood centers.[14,15]

The total number of apheresis PLT donations collected and the number of components manufactured was derived from the Red Cross centralized database. The proportion of procedures performed with different collection sets was calculated based on the purchased volume of collection sets for the system during the 3-year study period.

Detection of bacterial contamination

An automated microbial detection system was used for aerobic cultures according to manufacturer's instructions, as previously described.[12] The Red Cross, like the majority (85%) of licensed blood centers in North America, inoculated only an aerobic bottle, although an additional anaerobic bottle was recommended, but not required, by the manufacturer. This decision was based on our assessment of the data supporting the practice.[16,17] PLT components were sampled at least 24 hours after collection, divided into two cocomponents if PLT yield was greater than 6.5×10^{11} PLTs and released into inventory for distribution 12 hours after initiation of culture, if cultures were negative. Cultures were continued for the 5-day shelf life of the PLT components. All components associated with positive initial culture results were quarantined or retrieved if already distributed to transfusion services. A second 4- to 5-mL sample was taken from these initially positive components or cocomponents and inoculated into a new aerobic bottle for confirmatory culture. The initial and subsequent positive culture bottles were sent to independent microbiology laboratories for bacterial isolation and identification. A confirmed-positive result is defined as the growth of the same organism in the initial and confirmatory sample; a false-positive result is defined as a positive bottle signal but a negative result on subsequent culture, which was further characterized as either possible sampling contamination or instrument error.[12] If PLT components were not available for confirmatory culture, because they were transfused or destroyed during the manufacturing process, the initial positive signal could not be resolved and the results were classified as "indeterminate."

Septic transfusion reactions

All transfusion reactions reported to American Red Cross blood centers were investigated by regional physicians and compiled in a centralized database. Clinical criteria for a possible septic transfusion reaction to apheresis PLTs were any of the following symptoms within 4 hours of transfusion: fever of greater than 39°C or a change of greater than 2°C from pretransfusion value, rigors, tachycardia greater than 120 bpm or a change of more than 40 bpm from pretransfusion value, or an increase or decrease of more than 30 mmHg in blood pressure.[8] A definite septic transfusion reaction was defined by clinical criteria associated with a culture-positive residual component and a culture-positive recipient demonstrating the same bacteria as determined by antibiotic sensitivity and/or pulsed-field gel electrophoresis or by clinical criteria in both recipients of proven culture-positive cocomponents. A probable septic transfusion reaction was defined by clinical criteria and a positive culture on the residual component without matching positive culture results in the

recipient or clinical criteria and matching culture results in two different recipients of each cocomponent that was not cultured. For the risk analysis, the probable and definite septic reactions were combined and the generic term "septic reactions" denotes the sum of these reports. Possible septic reactions that did not meet the criteria of probable or definite reactions were excluded.

Statistical analysis

Rates of culture-positive reactions were compared with chi-square statistics with Yates correction and adjustment for multiple comparisons (5 × 2 table, degrees of freedom [df] = 4).[18] Odds ratios (ORs) and 95 percent confidence intervals (CIs) were computed to compare the odds of contamination with collections performed utilizing one-arm versus two-arm procedures.[19] A p value of less than 0.05 was considered significant. The American Red Cross Institutional Review Board reviewed and approved this study.

RESULTS

Characterization of positive results from the bacterial detection system

From March 1, 2004, to May 31, 2006, the American Red Cross performed routine QC testing for bacterial detection on 1,004,206 apheresis PLT donations from approximately 150,000 donors, which yielded 1,496,134 components for distribution for transfusion. Culture results of 186 donations were confirmed positive for bacterial contamination with 188 organisms (2 cultures contained 2 species each). The overall rate for confirmed-positive cultures was 18.5 in 10^5 donations (1:5,399); false-positive cultures, 34.7 in 10^5 donations (1:2,886); and indeterminate, 7.8 in 10^5 donations (1:12,874) (Table 1). Of the total donations, 61 percent were collected by a two-arm procedure (46%, Amicus; 15%, Spectra) and 39 percent by a one-arm procedure (12%, Amicus; 7%, Spectra; 20%, Trima) (Table 2). A significant difference was noted in the rates of confirmed-positive cultures (confirmed-positive cultures, df = 4, $\chi^2 = 16.6$; p = 0.002; Fig. 1), but not false-positive cultures (false-positive cultures, df = 4, $\chi^2 = 7.1$; p = 0.1) among the five collection

TABLE 1. All positive bacterial culture results from 1,004,206 apheresis PLT donations tested between March 1, 2004, and May 31, 2006

Result	Number (%)	Rate per 10^5 donations	Risk per donation
Confirmed-positive	186 (30.3)	18.5	1:5,399
False-positive			
Total	348 (57.0)	34.7	1:2,886
Sampling contamination	198 (32.4)	19.7	1:5,072
Instrument signal error	150 (24.5)	14.9	1:6,695
Indeterminate	78 (12.7)	7.8	1:12,874
Total number of positive donations	612	60.9	1:1,641

TABLE 2. Confirmed-positive bacterial cultures by donation type

	Two-arm procedure			One-arm procedure				
	Amicus	Spectra	Total	Amicus	Spectra	Trima	Total	OR (95% CI)*
Total number of collections	467,090	150,505	617,595	117,830	70,223	198,558	386,611	
Likely skin contaminants								
Coagulase-negative *Staphylococcus*†	70	16	86	3	2	10	15	
Staphylococcus aureus	2	1	3	2	0	3	5	
Streptococcus sp.‡	10	2	12	3	2	4	9	
Micrococcus sp.	1	0	1	0	0	0	0	
Bacillus sp.	2	2	4	0	0	1	1	
Total number of skin contaminants	85	21	106	8	4	18	30	
Rate per 10^5 cultures	18.2	14.0	17.2	6.8	5.7	9.1	7.8	2.2 (1.5-3.3)
Nonskin organisms								
Streptococcus sp.§	6	4	10	2	1	3	6	
Escherichia coli	5	3	8	0	1	3	4	
Klebsiella sp.	6	0	6	0	0	1	1	
Listeria sp.	1	1	2	2	0	0	2	
Serratia sp.	4	0	4	1	0	0	1	
Other‖	4	0	4	2	0	0	2	
Total nonskin organisms	26	8	34	7	2	7	16	
Rate per 10^5 cultures	5.6	5.3	5.5	5.9	2.8	3.5	4.1	1.3 (0.7-2.4)
All confirmed-positive cultures	111	29	140	15	6	25	46	
Rate per 10^5 cultures	23.8	19.3	22.7	12.7	8.5	12.6	11.9	1.9 (1.4-2.7)

* ORs compare the rates of positive cultures for total one-arm vs. total two-arm procedures.
† Coagulase-negative *Staphylococcus* including *S. epidermidis, S. capitis, S. hemolyticus, S. saprophyticus,* and *S. lugdunensis.*
‡ α-Hemolytic *Streptococcus, S. viridans* group, *S. mitis/oralis, S. sanguis,* and *S. salivarius.*
§ β-Hemolytic *Streptococcus, S. agalactiae, S. bovis,* and *S. pneumonia.*
‖ *Enterococcus avium, Granulicatella adiacens, Citrobacter* sp., *Lactobacillus* sp., and *Enterobacter aerogenes.*

procedures (Table 3). There was no significant difference in the rates of confirmed-positive cultures among the three collection devices for the one-arm procedures or between the two collection devices for two-arm procedures ($p > 0.05$), which were therefore analyzed as distinct groups for comparison (one-arm vs. two-arm procedures; Fig. 1). The rate of confirmed bacterial contamination of apheresis PLTs was 1.9-fold greater for two-arm procedures compared to one-arm procedures (OR, 1.9; 95% CI, 1.4-2.7; Fig. 1 and Table 2). There were no significant differences in the rates of false-positive culture results, due to either possible sampling contamination or instrument error, for two-arm procedures compared to one-arm procedures (OR, 1.1; 95% CI, 0.9-1.4; Table 3).

Bacterial isolates that likely represent skin flora, including *Staphylococcus* spp., comprised the majority of confirmed-positive cultures (136 of 186 [73%]; Table 2), whereas the remainder (50 of 186 [27%]) were bacteria not typically found on skin surfaces that could reflect asymptomatic bacteremia in the donor.[20] Skin contaminants were more frequent in two-arm procedures (OR, 2.2; 95% CI, 1.5-3.3) compared to one-arm procedures, but there was no significant difference between procedure types for other bacteria (OR, 1.3; 95% CI, 0.7-2.4; Table 2).

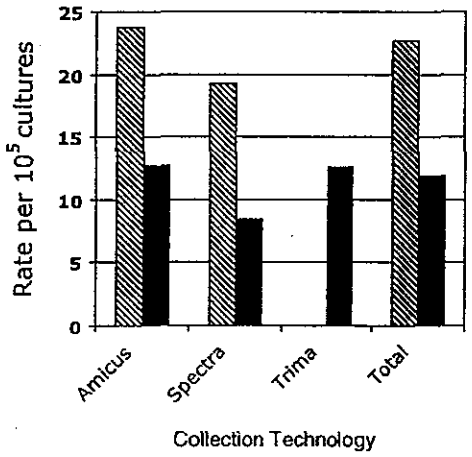

Fig. 1. The rate of confirmed-positive PLT cultures per 100,000 collections, analyzed by the technology utilized for collection, including the Baxter Amicus, Gambro Spectra, and Gambro Trima technologies and one-arm (■) and two-arm (▨) procedures.

Transfusion of culture-positive apheresis PLTs

Of the 612 donations that tested initially positive, 97 apheresis PLT components had been transfused at the time of the positive culture result, only one of which was associated with a confirmed-positive result (coagulase-negative *Staphylococcus*). The confirmed-positive component was transfused without reported recipient reaction on Day 2 after collection, and was detected 48 h after inoculation. No septic transfusion reactions were reported for the other recipients of apheresis components associated with false-positive or indeterminate results. The mean incubation time to a positive result for transfused components with false-positive or indeterminate culture results was 74 ± 30 hours. These data support the safety of the Red Cross protocol, which incorporated a 12-hour inventory hold period after inoculating the bacterial culture bottle. Operational constraints, including inventory release, distribution, and transport generally prevented transfusion of products within 24 hours of sample inoculation (data not shown).

Investigation of donors with confirmed-positive results

All donors with confirmed-positive bacterial culture results were investigated to evaluate a possible source of contamination. In one series of cases, *Listeria monocytogenes* was detected in three asymptomatic donors in different states during a 3-month time period. Investigation in collaboration with the Centers for Disease Control and Prevention revealed no common source. Donors of contaminated PLT products likely due to asymptomatic bacteremia were referred to their physician for further evaluation. One donor of a *Streptococcus bovis*–contaminated product was found to have an occult moderately differentiated adenocarcinoma, as previously reported[21] and another donor found with a positive

TABLE 3. False-positive cultures by donation type

	Two-arm procedure			One-arm procedure				
	Amicus	Spectra	Total	Amicus	Spectra	Trima	Total	OR (95% CI)*
Total number of collections	467,090	150,505	617,595	117,830	70,223	198,558	386,611	
False-positive (sampling contamination)	93	36	129	18	13	38	69	
Rate per 10^5 cultures	19.9	23.9	20.9	15.3	18.5	19.1	17.8	1.2 (0.9-1.6)
False-positive (instrument signal error)	77	19	96	7	11	36	54	
Rate per 10^5 cultures	16.5	12.6	15.5	5.9	15.7	18.1	14.0	1.1 (0.8-1.5)
All false-positive cultures	170	55	225	25	24	74	123	
Rate per 10^5 cultures	36.4	36.5	36.4	21.2	34.2	37.3	31.8	1.1 (0.9-1.4)

* ORs compare the rates of positive cultures for total one-arm vs. total two-arm procedures.

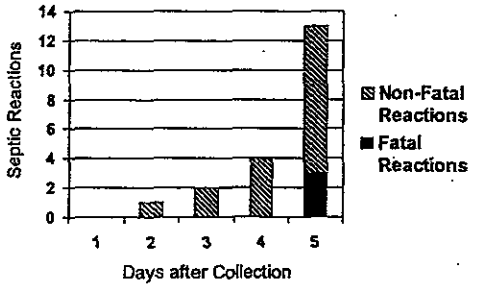

Fig. 2. Septic transfusion reactions analyzed by the interval between collection and transfusion. (■) Fatal reactions; (▨) nonfatal reactions.

culture for β-hemolytic *Streptococcus* G reported a diagnosis of colonic carcinoma 1 month after the involved collection. The donor investigation was unremarkable in most cases and the donors were allowed to continue to donate PLTs. No donor has been implicated in more than one episode of PLT contamination.

Clinical transfusion reactions to screened-negative apheresis components

From March 1, 2004, to May 31, 2006, a total of 20 definite or probable septic transfusion reactions, including 3 fatalities, were reported to the Red Cross, after transfusion of bacterially screened apheresis PLT components. More septic reactions occurred with older PLT units, and 13 of the 20 reactions including all 3 fatalities occurred after transfusion on Day 5 after collection (Fig. 2). During this period, a nationwide survey reported that the majority of apheresis PLT products were transfused on Day 3 or earlier (median, Day 3; mean, Day 3.08) after collection,[22] suggesting that the rate of septic reactions increases with PLT age. All of the implicated components had negative QC culture results after 5 days of culture indicating false-negative BacT/ALERT results. All collections were performed with the standard povidone-iodine–based skin preparation. Two-arm procedures were used for 15 implicated collections (17 septic transfusion reactions, including the 3 fatalities), and one-arm procedures for 2 implicated collections (3 septic transfusion reactions; Table 4). The risk of septic transfusion reactions after transfusion of screened-negative apheresis PLTs was 4.7-fold greater for two-arm collection procedures compared to one-arm procedures (OR, 4.7; 95% CI, 1.2-18.4). When analyzed by distributed components, PLTs from two-arm procedures tended to be 3.3-fold more likely to be involved in a reported septic reaction (OR, 3.3; 95% CI, 1.0-10.6). Components collected with two-arm procedures were implicated in the 11 definite septic transfusion reactions; 9 of the 11, including the 3 fatalities, were found with *Staphylococcus* spp. contamination.

One recipient fatality implicated *Staphylococcus lugdunensis* (coagulase-negative *Staphylococcus*) and has been described previously.[23] A second fatal case involved a 70-year-old woman with breast cancer who died shortly after receiving a PLT product stored for 5 days that was found to be contaminated with *Staphylococcus aureus*. The cocomponent had been transfused to a different patient on Day 3 of storage without reported reaction; however, subsequent chart review by the regional Red Cross physician revealed this patient developed fever 2 hours after transfusion with subsequent blood cultures demonstrating *S. aureus*. The bacterial isolates from both recipients and the residual apheresis PLT product implicated in the fatality had identical antibiotic sensitivity profiles and banding patterns on pulsed-field gel electrophoresis. The third fatal case involved a 68-year-old woman with multiple myeloma after autologous peripheral blood cell transplant and also implicated *S. aureus*. In contrast, eight of the donations implicated in septic reactions had an apheresis PLT cocomponent had been distributed and transfused without reported reaction in six cases or outdated in the hospital in two cases.

DISCUSSION

The rate of bacterial detection in apheresis PLT components in the American Red Cross was 1:5399 (18.5 per 10^5) donations after implementation of culture-based screening. Although this likely represents an important improvement in blood component safety, there remains an appreciable risk of transfusion-related sepsis or death after transfusion of screened-negative components. Utilizing data from passive surveillance of transfusion reactions reported to the Red Cross in the study period, this residual risk is estimated as at least 1:74,807 septic reactions and 1:498,711 fatalities per distributed component. Before the introduction of bacterial testing, comparable rates within the Red Cross system were estimated at approximately 1:40,000 and 1:240,000, respectively[12] (based on a 10-month period in 2003 when approximately 480,000 apheresis PLTs were distributed), an approximate 50 percent decrease in reported reactions and fatalities. The significance of this declining trend ($p = 0.11$) is unclear, given the likelihood of variable and incomplete reporting of cases in a passive surveillance system.

The bacterial contamination rate associated with two-arm procedures (1:4,411) was almost twice that observed for one-arm procedures (1:8,405; OR, 1.9; 95% CI, 1.4-2.7). Similarly, more than a fourfold increase in reported septic transfusion reaction rates was observed between two-arm procedures (1:41,173 collections) compared to one-arm procedures (1:193,305 collections; OR, 4.7; 95% CI, 1.2-18.4). These observations may reflect the fact that during one-arm procedures, the initial volume of

TABLE 4. Septic transfusion reactions to bacterial screen–negative apheresis PLTs (false-negative cultures) by donation type

	Two-arm procedure			One-arm procedure				
	Amicus	Spectra	Total	Amicus	Spectra	Trima	Total	OR (95% CI)*
Total number of collections	467,090	150,505	617,595	117,830	70,223	198,558	386,611	
Total number of distributed components	725,105	218,233	943,338	182,918	101,823	268,054	552,796	
Definite								
Coagulase-negative *Staphylococcus*	5	0	5	0	0	0	0	
Staphylococcus lugdunensis†	1	0	1	0	0	0	0	
Staphylococcus aureus†	3‡	0	3	0	0	0	0	
Enterobacter aerogenes	2‡	0	2	0	0	0	0	
Probable								
Coagulase-negative *Staphylococcus*	3	1	4	0	0	1	1	
Staphylococcus intermedius	0	0	0	0	2‡	0	2	
Enterococcus fecalis	0	1	1	0	0	0	0	
Pseudomonas fluorescens	1	0	1	0	0	0	0	
Total number of septic reactions by collections‡	13	2	15	0	1	1	2	
Rate per 10^5 collections	2.8	1.3	2.4	0.0	1.4	0.5	0.5	4.7 (1.2-18.4)
Total number of septic reactions by components	15	2	17	0	2	1	3	
Rate per 10^5 components	2.1	0.9	1.8	0.0	2.0	0.4	0.5	3.3 (1.0-10.6)

* ORs compare the rates of septic reactions for total one-arm vs. total two-arm procedures.
† Organisms implicated in three recipient fatalities.
‡ In three cases, the components from a single PLT collection were implicated in two separate septic reactions.

blood that may be contaminated with skin organisms during phlebotomy is separately collected for viral marker testing and does not enter the apheresis product. In contrast, the configuration with the two-arm procedures performed during this study collected the sample for testing from the return line, not the inlet (draw) line, which may have allowed blood contaminated during inlet line phlebotomy to enter the collection set and PLT product. Removal of the initial 10 to 40 mL of blood after venipuncture before drawing donors' blood has been shown to reduce the bacterial load introduced into manufactured blood components by 40 to 90 percent.[24-28] The clinical importance of reducing the probability of contamination is supported by data from a hemovigilance program that showed a concomitant reduction in septic transfusion reactions, which were almost all associated with skin organisms, after implementation of a 40-mL diversion pouch for whole blood–derived PLTs.[29] The alternative explanation, that increased risk with two-arm procedures simply reflects an additional needle stick, is unlikely as blood from the return line in two-arm procedures does not generally enter the apheresis machine to contaminate the apheresis product.

Based on the current analysis and on recently published data, the American Red Cross has converted to Amicus two-arm procedures which utilize an inlet line diversion pouch system and will phase out the use of the Spectra system. Although the Spectra two-arm procedure was not associated with an increase in septic reactions in our data, the twofold increased rate of true-positive bacterial cultures with this technology raises concern. The volume of product cultured in the BacT/ALERT system will be doubled (from 4 to 8 mL), which is predicted to increase QC culture sensitivity by up to 25 percent.[30] This combination of interventions is predicted to reduce the rate of septic transfusion reactions described in this report by greater than 75 percent and efficacy will be monitored prospectively. Further strategies to reduce contamination may include additional modification of culture volume or conditions, improved skin decontamination procedures,[15,24,31] introduction of point-of-issue retesting of products, and prospective pathogen reduction of apheresis blood components,[32] although the latter two options are not yet available as FDA-approved technologies in the United States.

Our data raise the question as to why the BacT/ALERT culture system has a significant false-negative rate when used as a QC test for apheresis PLTs. This system was validated for testing apheresis PLTs by the manufacturer utilizing in vitro spiked products, on the assumption that the detectable concentration of bacteria was in the range of 1 to 10 colony-forming units (CFUs) per mL and that organisms enter a log phase of growth to reach these levels during the 24-hour hold period before sampling.[33,34] Bacterial contamination of apheresis products at collection, however, may be as low as 1 to 10 CFUs per bag (0.003-0.03 CFUs/mL). At these low concentrations, bacteria may behave in an idiosyncratic fashion such that the duration of the lag phase of growth is unpredictable. The BacT/ALERT system has not been validated to reliably detect bacteria in this clinically relevant range and a residual risk of contamination almost certainly remains, especially in

older PLT units where low initial concentrations of bacteria with a prolonged lag phase that are missed due to sampling limitations may reach significant concentrations by the time of transfusion.

Division of the PLT sample between an anaerobic and aerobic bottle in the BacT/ALERT system has been recommended by the manufacturer to increase bacterial culture sensitivity and is currently under investigation in the PASSPORT protocol for extended storage PLTs.[13] The anaerobic culture bottle may detect obligate anaerobes that occasionally contaminate PLT products[9,35] as well as increase the sensitivity and decrease the time required for detection of some facultative aerobic organisms, including *Staphylococcus* sp. and *Streptococcus* sp.[36] Anaerobic testing, however, increases product losses and hospital recalls due to increased false-positive culture results and substantially increases the cost per test performed, due to the additional costs of the anaerobic bottle and the corresponding increase in the number of incubators required. Early studies in the United States have failed to demonstrate increased sensitivity for common PLT contaminants in routine practice.[37] Interpretation of European studies is confounded by poor differentiation between true- and false-positive initial culture results; however, the data support the view that the volume of product cultured, not necessarily the culture conditions, is an important determinant of culture sensitivity for common aerobic PLT contaminants.[27] More rapid detection of bacterial contamination will likely have little impact on safety, because our data demonstrate that septic reactions are associated with false-negative culture results that do not become positive even after prolonged incubation. We also describe 97 instances where delayed initial-positive culture results allowed PLT products to be transfused before detection. Earlier detection of bacterial growth may have prevented these transfusions; however, none of these cases were associated with a septic transfusion reaction.

Obligate anaerobes such as *Clostridium* sp. and *Propionibacterium acnes* are likely skin contaminants, which may be more effectively mitigated by diverting the initial sample from the draw line (e.g., sample first/diversion pouch strategies) than detection strategies that have inherent sampling and sensitivity limitations.[9,35,38] *Clostridium* spp. and *Eubacterium* spp., in particular, are anaerobes that have been associated with fatalities.[35,39,40] The detection of *Clostridium* sp. has been validated in pooled whole blood–derived PLTs; however, at spiking concentrations of 0.2 CFUs per mL, the BacT/ALERT system failed to detect contamination in 7 of 10 samples tested.[41] At higher concentration (1.9 CFU/mL), *Clostridium perfringens* was reliably detected in 10 of 10 anaerobic bottles.[41] The authors are not aware of instances where *Clostridium* sp. or *Eubacterium* sp. have been detected by anaerobic bottles in routine apheresis PLT cultures.[9,27,37,42] Interestingly, there are reports of both *P. acnes* and *Clostridium* sp. detection in aerobic BacT/ALERT bottles.[43,44] These observations raise the possibility that the clinically significant anaerobes are those that grow as facultative aerobes in the aerobic conditions of PLT storage.

Reports of septic reactions and fatalities persist despite the implementation of both aerobic and anaerobic cultures: de Korte and coworkers[27] report two cases of sepsis caused by units contaminated with *Bacillus cereus* bacteria and recently Schmidt and coworkers[45] reported both a fatality and a severe septic reaction case caused by the two split units of an apheresis product later found to be contaminated with *Klebsiella pneumoniae* bacteria. These data, and our finding that septic reactions and fatalities are more likely with PLTs transfused on Day 5 after collection despite the report that the majority of PLTs are transfused on Day 3 or earlier,[22] now raise concerns about an increasing residual risk of sepsis with PLT storage age and the safety of extending PLT storage beyond 5 days.

Finally, bacterial screening of apheresis PLT donors has provided a unique opportunity for a secondary public health benefit. Donor centers investigating bacterial cultures and recipient reactions have recommended medical referral for donors with possible asymptomatic bacteremia, which in rare cases has led to early identification of serious medical conditions, including adenocarcinoma of the colon,[21,46] bacterial endocarditis,[2] and subclinical osteomyelitis.[47] Most importantly, as demonstrated in this report, the ongoing surveillance for bacterial detection among blood donors allowed for the identification of a possible design flaw in two-arm collection procedures. Bacterial contamination remains a serious risk of PLT transfusion despite bacterial culture testing, emphasizing the need for the appropriate clinical use of PLT transfusions and ongoing vigilance to recognize, investigate, promptly treat, and report all suspected septic transfusion reactions.

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研究報告の概要

高病原性鳥インフルエンザウイルス(H5N1)を含むインフルエンザウイルスが、(輸血用)血液の脅威となる可能性がある。当研究チームはミニプール核酸増幅法を用いて 10,272 名の血液ドナーの検体を解析した。この検査法の測定感度は、一般的インフルエンザウイルス用プライマーについては 804 geq/ml で、インフルエンザ(H5N1)サブタイプ特異的プライマーでは 444 geq/ml であった。本研究は、インフルエンザウイルスに対して、このようなスクリーニング検査が可能であることを示した。
血液製剤の安全性を確保するため、このスクリーニング法を、世界的流行の際に遅滞なく血液スクリーニング手順に導入できるであろう。今回のインフルエンザスクリーニング方法の感度は、現在報告されている感染者血漿でのウイルス濃度を検知するのに十分であると考えられる。しかし、すべてのミニプール法と同様、ドナーのウイルス血症レベルがスクリーニングアッセイ法の測定感度以下であるために(スクリーニング検査で除外されず)稀に感染が輸血レシピエントに伝播されることがありうる。
こうしたリスクを減少させるため、米国でウエストナイルウイルススクリーニングに導入されているように、selective infectious dose NAT strategy (例えば、標準のミニプールスクリーニングアルゴリズムによって、週当たり少なくとも 1 件のウイルス血症血液が採取された場合に、infectious dose NAT 検査を開始する)が必要となるかもしれない。

使用上の注意記載状況・その他参考事項等

代表として献血ヴェノグロブリン-IH ヨシトミの記載を示す。
2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT（GPT）値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）及び pH3.9～4.4 の条件下での液状インキュベーション処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
高病原性トリインフルエンザ A (H5N1) ウイルスを含むインフルエンザウイルスに対するスクリーニング検査が、ミニプール NAT により実施可能であることが示されたとの報告である。 血漿分画製剤からの高病原性トリインフルエンザA（H5N1）ウイルス伝播の事例は報告されていない。また、万一原料血漿に高病原性トリインフルエンザA（H5N1）ウイルスが混入したとしても、BVDをモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

Blood Screening for Influenza

Michael Kai Hourfar,* Anna Themann,* Markus Eickmann,† Pilaipan Puthavathana,‡ Thomas Laue,§ Erhard Seifried,* and Michael Schmidt*

Influenza viruses, including highly pathogenic avian influenza virus (H5N1), could threaten blood safety. We analyzed 10,272 blood donor samples with a minipool nucleic acid amplication technique. Analytical sensitivity of the method was 804 geq/mL and 444 geq/mL for generic influenza primers and influenza (H5N1) subtype–specific primers. This study demonstrates that such screening for influenza viruses is feasible.

In the 20th century, 3 influenza-related pandemics occurred (1918 Spanish influenza, 1957 Asian influenza, and 1968 Hong Kong influenza) (*1*), which are now known to represent 3 different antigenic subtypes of the influenza A virus: H1N1, H2N2, and H3N2. Major influenza epidemics show neither periodicity nor a predictable pattern, and all differ from one another. Evidence suggests that true pandemics involving changes in hemagglutinin subtypes are caused by genetic reassortment in animal influenza A viruses. Since 2003, the World Health Organization has reported the infection of ≈218 persons and 124 deaths (56.9%; as of May 23, 2006) caused by the (H5N1) subtype in 10 different countries; a probable person-to-person transmission of the avian influenza virus was suggested (*2*). Most countries predicted death rates of 14–1,685 persons per 100,000 population in the event of a pandemic and estimated that up to 2,707 persons per 100,000 population would become infected (*3*).

Our study demonstrates that screening donor blood for influenza A (H5N1) subtype or for influenza viruses in general by minipool nucleic acid amplification technique (NAT) is feasible. To ensure the safety of blood products, this screening technique could be introduced into the blood-screening procedure without delay in the case of a pandemic.

The Study

To increase blood safety, we introduced minipool NAT screening in our blood donor service in 1997 for hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV-1 and in 2000 for hepatitis A virus (HAV) and parvovirus B19 (*4*). For these purposes, 100-µL aliquots of up to 96 blood samples were pooled. The complete pool of up to 9.6 mL was centrifuged at 58,000× *g* for 60 min at 4°C. Viruses were extracted by using spin columns, and nucleic acid was eluted in a total volume of 75 µL. Only 60 µL of extract is needed for routine NAT screening. A residual volume of 15 µL can then be used for additional NAT testing (*5*) for influenza viruses.

The real-time quantitative amplification of influenza/H5 was performed according to the manufacturer's instructions (Artus Influenza/H5 LC RT-PCR Kit, QIAGEN, Hamburg, Germany) by using a thermocycler (LightCycler; Roche Applied Science, Mannheim, Germany). The test consists of 2 individual amplification reactions. In the first step, a generic influenza PCR is performed. The specificity of this reaction was demonstrated for all subtypes of influenza A (H1–H15, N1–N9) and all subtypes of influenza B. Samples with a positive test result in the first PCR were analyzed in a second PCR with influenza (H5N1)–specific primers and probes. Therefore, the assay allows differentiation between avian influenza (H5N1) and other influenza virus strains.

To mimic a situation like an H5-positive donation, a purified culture supernatant of Vero cells infected with influenza (H5N1) (strain A/Thailand/1 (KAN-1)/2004) (*6*) was used as an external quantification standard. Virion integrity in this preparation was confirmed by electron microscopy. The viral RNA concentration was determined in an external laboratory by multiple quantitative real-time PCR determinations (*7*). Different dilutions of the external influenza (H5N1) subtype quantification standard (0.0, 0.91, 1.96, 3.91, 7.81, 15.63, 31.25, 62.5, 125, and 250 PFU/mL) were prepared, and 100 µL of each dilution was spiked into 9.5-mL negative plasma pools. Each dilution was repeatedly spiked and tested in 8 minipools. Five microliters of the extract was analyzed with the generic influenza NAT as well as with the specific influenza (H5N1) NAT. Results are shown in Tables 1 and 2. Probit analysis of these data yielded a detection probability of >95% in parallel tests when an average of at least 13.4 PFU/mL (95% confidence interval [CI] 8.3–184 PFU/mL) and 7.4 PFU/mL (95% CI 5.2–14.7 PFU/mL) for influenza generic assay and for the influenza (H5N1)–specific test, respectively, were present in individual plasma samples before pooling.

A total of 117 routine minipools, representing 10,272 blood donor samples, containing an average of 88 ± 8 samples per pool, had previously been tested for HIV-1, HBV, HCV, HAV, and parvovirus B19. All pools were negative for influenza virus when tested with the generic influenza PCR and the influenza (H5N1)–specific PCR. One pool had invalid results (failed amplification of internal control RNA, representing 0.01% of all analyzed runs).

*German Red Cross, Frankfurt, Germany; †Phillips University, Marburg, Germany; ‡Mahidol University, Bangkok, Thailand; and §QIAGEN, Hamburg, Germany

Table 1. Analytical sensitivity for influenza virus in plasma samples*

PFU (H5N1) spiked in minipools	No. positive/ no. tested	% Positive
250.0	8/8	100
125.0	8/8	100
62.5	8/8	100
31.3	8/8	100
15.6	7/8	87.5
7.8	8/8	100
3.9	4/8	50
0.0	0/8	0

*Influenza (H5N1) standard was extracted from 9.6 mL of 96 pooled donor samples after centrifugation. Five microliters of 75-µL nucleic acid extract was analyzed. The 95% detection limit was 13.4 PFU/mL; the 50% detection limit was 4.8 PFU/mL.

Conclusions

As reported by AuBuchon et al. (*8*), NAT significantly increased the safety of blood products. At the German Red Cross, look-back examinations showed only 1 transfusion had transmitted HIV-1 (1998) after the introduction of NAT testing. Blood donor screening by NAT was made technically and financially feasible by creating minipools of up to 96 individual samples per pool. Roth et al. demonstrated an efficient enrichment for all tested viruses in plasma samples (*9*). In the absence of an infective donor, different concentrations of the new influenza genotype H5N1 were spiked into minipools of 95 samples. As shown in Table 2, the influenza (H5N1) subtype was detected by the generic influenza primers as well as by the influenza (H5N1)–specific primers when our routine minipool screening procedure was used. Sensitivity was expressed as PFU/mL and can be converted into viral genome copy number according the calculation of Yoshikawa et al. (*7*). Therefore, the analytical sensitivity was ≈804 geq/mL and 444 geq/mL for a generic influenza and for the influenza (H5N1) subtype, respectively.

After screening 10,272 samples by minipool NAT, none of the samples were found to be infected by influenza, which corresponds with the low EISS Index (European Influenza Surveillance Scheme index) of <20 during the study period (February–April, 2006) (*10*). An EISS index >80 is expected during an influenza epidemic, as was seen in 2005. Therefore, blood screening should be repeated during the next acute influenza season.

Table 2. Analytical sensitivity for avian influenza (H5N1) virus subtype for plasma samples*

PFU (H5N1) spiked in minipools	No. positive/ no. tested	% Positive
125.0	8/8	100
62.5	8/8	100
31.3	8/8	100
15.6	8/8	100
7.8	7/8	87.5
3.9	7/8	87.5
1.9	4/8	50
0.9	3/8	37.5
0.0	0/8	0

*Influenza (H5N1) standard was extracted from 9.6 mL of 96 pooled donor samples after centrifugation. Five microliters of 75-µL nucleic acid extract was analyzed. The 95% detection limit was 7.4 PFU/mL; the 50% detection limit was 2.5 PFU/mL.

Accepted incubation periods for influenza range from 2 to 10 days (*11,12*). As with other viruses, a viremic phase of infection can be assumed to precede clinical symptoms such as fever (*13,14*). Recently Chutinimitkul et al. (*15*) detected influenza (H5N1) virus (3,080 copies/mL) in the plasma of a 5-year-old boy, which indicates a viremic phase of influenza (H5N1) infection. Those donors may be infective, especially to immunosuppressed patients. In addition to quarantine of infected patients, treatment with antiviral drugs, and development of avian influenza vaccines, blood donors should be tested during a pandemic to avoid transfusion-transmitted infections. Our study demonstrates that NAT screening could be incorporated into blood testing without delay and that the influenza virus could be sufficiently enriched by centrifugation. Sensitivity of our influenza-screening method would have been sufficient to detect recently reported virus concentrations in plasma of infected persons (*15*). However, as with all minipool methods, infections can be transmitted to transfusion recipients on rare occasions because the viremia level in the donor is below the analytical sensitivity of the screening assay.

To reduce this risk, a selective infectious dose NAT strategy (e.g., triggering of infectious dose NAT testing when at least 1 viremic donation is collected per week with the standard minipool screening algorithm), as performed for West Nile virus (WNV) screening in the United States might be necessary. Implementation of WNV-NAT in the United States in 2003 interdicted well over 1,000 donations from persons infected with WNV and is a good example of successful implementation of NAT screening for emerging viruses.

The collective fight against new viruses such as severe acute respiratory syndrome virus, WNV, or influenza (H5N1) presents an immense challenge for the whole community, but new molecular-biologic methods offer opportunities to overcome this challenge. NAT screening tests are now available soon after the sequencing of new viruses. In the absence of a general pathogen inactivation method for all blood products (erythrocytes, platelets, and plasma), the NAT screening procedure allows testing for new viruses to ensure blood safety.

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Mr Hourfar is a research scientist at the German Red Cross blood donor service. His research interests include the molecular epidemiology of pathogens with special focus on blood safety.

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Address for correspondence: Michael Schmidt, Institute of Transfusion Medicine and Immunohematology, Johann Wolfgang Goethe University, German Red Cross, Sandhofstr. 1, 60528 Frankfurt am Main; Frankfurt; Germany; email: mschmidt@blutspende.de

番号：2

別紙様式第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 平成19年9月13日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	Yang Yang, et al. Detecting Human-to-Human Transmission of Avian Influenza A (H5N1) Emerg Infect Dis Vol.13, No.9, September 2007;1348-53	公表国 米国	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要

高病原性鳥インフルエンザA(HPAI)サブタイプH5N1は、東南アジアの広範囲に渡ってヒトからヒトへの感染による家族クラスターを引き起こした。このウイルス（または別の人畜共通インフルエンザウイルス）が、ヒトからヒトへの感染を維持する能力を得ている場合、世界的大流行を引き起こす恐れがある。
インドネシアのスマトラ北部とトルコ東部でHPAI（N5H1）感染した家族クラスターが、ヒトからヒトへの感染によるものであったかどうかを調べるため統計的手法を使用した。ヒトからヒトへの感染が起こると仮定し、地域basic reproductive number(R0)と二次感染の発生率(SARs)を推測する。解析の結果、トルコ(p=0.114)ではなくスマトラ（p=0.009）の家族クラスターで、ヒトからヒトへの感染した統計的証拠を見出した。スマトラでは家族内SAR（二次感染発生率）は0.29（95%信頼区間：0.15～0.51）であった。地域R0の推定下限値は1.14（95%信頼区間:0.61～2.14）であった。HPAI（H5N1）監視効果、封じ込め対応、そして実地評価には、ウイルス株の大流行の可能性阻止と監視が不可欠である。

報告企業の意見	今後の対応
高病原性トリインフルエンザ（H5N1）が「人から人へ感染した」ことを、統計的手法によって証明した初めての報告である。本報告は、新たに判明した感染経路に関するもの且つ重大な感染症に関するものと判断する。	本研究報告は、ヒト血液を原料とする血漿分画製剤とは直接関連するものではなく、現時点で特に当該生物由来製品に関し措置等を行う必要はないと判断する。本研究報告では、ウイルスが伝播するためにはヒトとの極めて密接な接触が必要だったとし、簡単に伝播するわけではないことを示唆しているが、このウイルスがヒトからヒトへの感染を維持する能力を有したかどうかは不明と報告しており、今後も同様の情報に注意し収集する必要があると考える。

使用上の注意記載状況・その他参考事項等

特になし

49

MedDRA Version(10.1)

DRL 2007-00

Detecting Human-to-Human Transmission of Avian Influenza A (H5N1)

Yang Yang,* M. Elizabeth Halloran,*† Jonathan D. Sugimoto,*† and Ira M. Longini, Jr.*†

Highly pathogenic avian influenza A (HPAI) subtype H5N1 has caused family case clusters, mostly in Southeast Asia, that could be due to human-to-human transmission. Should this virus, or another zoonotic influenza virus, gain the ability of sustained human-to-human transmission, an influenza pandemic could result. We used statistical methods to test whether observed clusters of HPAI (H5N1) illnesses in families in northern Sumatra, Indonesia, and eastern Turkey were due to human-to-human transmission. Given that human-to-human transmission occurs, we estimate the infection secondary attack rates (SARs) and the local basic reproductive number, R_0. We find statistical evidence of human-to-human transmission ($p = 0.009$) in Sumatra but not in Turkey ($p = 0.114$). For Sumatra, the estimated household SAR was 29% (95% confidence interval [CI] 15%–51%). The estimated lower limit on the local R_0 was 1.14 (95% CI 0.61–2.14). Effective HPAI (H5N1) surveillance, containment response, and field evaluation are essential to monitor and contain potential pandemic strains.

Highly pathogenic avian influenza A (HPAI) subtype H5N1 is repeatedly crossing the species barrier to humans. Since December 2003, a total of 291 cases of HPAI (H5N1) have been reported in humans, resulting in 172 deaths (i.e., 59% case-fatality ratio) in 12 countries, mostly in Southeast Asia (*1*). Among these cases, 31 family clusters have been documented, ranging in size from 2 to 8 family members. How many of these clusters are due to a common avian source and how many are due to human-to-human transmission are important facts to determine. Should one of these HPAI (H5N1) strains gain the capacity for sustained human-to-human transmission, the resulting outbreak, if not contained, would spread worldwide through the global transportation network more rapidly than adequate supplies of vaccine matched to the new variant could be manufactured and distributed (*2,3*). We analyzed data from 2 of the largest of the familial clusters to ascertain if human-to-human transmission took place, and if so, how transmissible the strain was.

*Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; and †University of Washington, Seattle, Washington, USA

Methods

May 2006 Human Avian Influenza Family Cluster, Indonesia

During late April and early May 2006, a cluster of 8 cases of HPAI (H5N1) was detected and investigated by the Indonesian public health surveillance system in northern Sumatra (*4–6*). All case-patients were members of the same extended family. Seven of them resided within 3 adjacent houses in the village of Kubu Sembilang. The remaining patient resided with his immediate family in the village of Kabanjahe (≈10 km away).

The index patient was a 37-year-old woman, thought to have been exposed to dead poultry and chicken fecal material before onset of illness. She also reportedly maintained a market stall that sold live chickens. Although her illness was not confirmed to have been caused by avian influenza (H5N1), her death on May 5, 2006, is suspected to be the result of HPAI (H5N1) infection because of her reported symptoms, illness progression, and prior contact with diseased or dead poultry.

Twenty members of her extended family are suspected to have been in contact with her, many during a family gathering on April 29, 2006 (*7*). At that time, she was manifesting symptoms (i.e., she had a heavy cough, was severely ill, and was prostrate). That night, 9 of these members slept in the same small room as she did (indicated by a black triangle in online Appendix Figure 1, available from www.cdc.

gov/EID/content/13/9/1348-appG1.htm). Of these 9 family members, 2 of her sons (15 and 17 years of age) and her 25-year-old brother, who lived in Kabanjhe, became ill in the next 3 weeks. The sons died. The brother was the only person from this family cluster to recover.

Of the remaining 11 family members, 4 became ill and died. The 29-year-old sister of the index patient, who lived in an adjacent house, became ill after she provided direct personal care to her ill sister (*7*). The 18-month-old daughter of this sister also became ill after she was in the presence of the index patient with her mother. The 10-year-old nephew of the index patient, who lived in the other house adjacent to hers, became ill after he attended the family gathering and frequently visited his aunt's house. The nephew's father became ill after he personally cared for his son. The possibility that HPAI (H5N1) was transmitted from the nephew to his father is also supported by genetic sequencing data (*4*). Though symptoms did not develop in the mother of the nephew, she was directly exposed to her husband during his illness. All case-patients, except for the index patient, were confirmed as influenza (H5N1) positive by PCR. The nephew's mother was confirmed as influenza (H5N1) negative. As an intervention, 54 surviving relatives and close contacts were identified and placed under voluntary quarantine (*7*). All of these persons, except for pregnant women and infants, received oseltamivir prophylactically.

December 2005 Human Avian Influenza Family Cluster, Eastern Turkey

From December 18, 2005, (*8*) to January 15, 2006 (*9*), a cluster of 8 confirmed influenza (H5N1) cases was detected in Dogubayazit District in eastern Turkey (online Appendix Figure 2, available from www.cdc.gov/EID/content/13/9/1348-appG2.htm) (*10–13*). These case-patients were among 21 members of 3 households located within 1.5 km of each other (*14*). All confirmed case-patients were hospitalized after onset of symptoms (*9*). Four of the confirmed case-patients died; the other 4 recovered (*9*). Ten of the remaining 14 household residents were hospitalized with avian influenza-like symptoms but were never confirmed to be infected with influenza (H5N1) (*9*). All but one of the hospitalized residents were children (6–15 years of age) (*9*).

Before onset of symptoms, 4 children from 1 household, 3 of whom had confirmed cases (including the index patient), were reported to have had close contact with the dead bodies of sick chickens (*15*). The 2 confirmed case-patients in the second household reportedly slaughtered a duck together on January 1, 2006, at the beginning of a die-off in the household's flock (*14*). Two of the remaining confirmed case-patients lived in the third household and had no history of contact with sick or dying poultry. The remaining confirmed case occurred in a fourth residence located near the first household (*10*), but because we lacked information on the number of household members and the case-patient's exposure history, we excluded it from these analyses. Most, if not all, of the 21 residents attended a dinner hosted by the family of the index patient on December 24, 2006, while he was symptomatic (*8*).

Statistical Methods

We used a previously developed statistical transmission model (*16,17*) to test whether human-to-human transmission occurred, and if it did, to estimate transmission parameters. In the model, persons mix with one another in households and between households. In addition, we include a common source of infection due to zoonotic exposure. Mathematical and statistical details are given in the online Technical Appendix (available from www.cdc.gov/EID/content/13/7/1348-Techapp.pdf).

Model of Probability of Transmission

We define p_i as the probability that an infectious household member infects another household member in 1 day. If the distribution of the infectious period is known, we can obtain the household secondary attack rate (SAR_1) from p_1, defined as the probability that an infectious household member infects another household member over his or her infectious period. Similarly, we define the daily transmission probability (p_2) and the community SAR (SAR_2) for between household spread. Finally, we define the daily probability (b) that any person is infected from a zoonotic source. The contact structure used for parameter estimation is shown in the Figure. We assume that the distributions of the incubation and infectious periods are predetermined by the investigator.

We establish the likelihood function for each person and then for the whole population for statistical inference. The likelihood function for a person is equivalent to the probability of observing the realized data on that person throughout the outbreak. The likelihood function for a person labeled i is built with the following steps: 1) Obtain the probability that person i is infected by an infectious source labeled j on day t, given person i is not infected up to day $t-1$. If source j is a person, this probability is p_1, for the same household, or p_2 for exposure in the community, multiplied by the probability of person j being infectious on day t. The probability of person j being infectious on day t is derived from the symptom-onset day of person j and the distribution of the infectious period. If source j is zoonotic, the infection probability is b. The probability of escaping infection is simply 1 minus the corresponding probability of infection. 2) Take the product of the probabilities obtained in step 1 over all humans and zoonotic sources j to obtain the probability of person i escaping infection by any

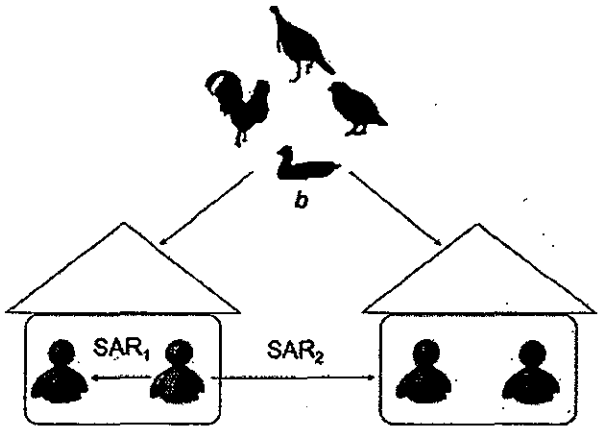

Figure. Schematic of estimation method. An infectious person (in red) infects a susceptible person (in green) in the same household with probability of household secondary attack rate (SAR_1) and infects a susceptible person in a different household with probability SAR_2. The common infectious source (i.e., avian hosts) infects a susceptible person with probability b per day. The likelihood function is constructed from symptom-onset dates and exposure information to estimate the above parameters

infectious source on day t. 3) Take the product of the probabilities obtained in step 2 over all days before and including day t to obtain the probability of person i escaping infection up to day t. 4) If person i is not infected by the end of the outbreak, the likelihood function for person i is the product of the probabilities of person i escaping infection up to the last day of observation. 5) If person i is observed to have symptom onset on day $\tilde{t}$ and the infection time is known to be t, the probability of the data regarding person i is the product of 3 pieces of information: a) the probability of person i escaping infection up to day $t-1$, b) the probability that person i is infected on day t, and c) the probability that the duration of the incubation period is $\tilde{t}-t$. Because we do not observe the infection time, the likelihood function for person i is obtained by summing the above product, a – c, over all potential values of t.

The likelihood function for the whole population is the product of all the individual likelihood functions. In the event that human-to-human transmission occurs, SAR estimates are used to estimate the local basic reproductive number (R_0), which is defined as the average number of secondary cases infected by a typical index case-patient in the beginning of the outbreak (online Technical Appendix). There is potential for sustained transmission if R_0 is >1. If human-to-human transmission is determined to be occurring, then the above parameters are estimated from the symptom dates and contact information from the population under study. Data on exposed persons who do not become ill form an important component of the inference procedure.

Statistical Test

We set up a statistical test with the null hypothesis being that no human-to-human transmission occurs, that is, $p_1 = p_2 = 0$. The alternative hypothesis is either p_1 or p_2 is not equal to 0, or both are not equal to zero. The test statistic we use is proportional to the ratio of the maximum value of the likelihood function assuming the null hypothesis is true (null likelihood) and the maximum value of the likelihood function at the estimated parameter values (full likelihood).

Specifically, we define the likelihood ratio test statistic as –2 log (the null likelihood function divided by the full likelihood function). If no human-to-human transmission occurs, the 2 likelihood functions would be roughly equal, and we expect to see a likelihood ratio close to 1, and, thus, a likelihood ratio statistic close to 0. A large value of the likelihood ratio statistic is evidence of deviation from the null hypothesis. The question is how to obtain a reference set of the likelihood ratio statistic values that we would see under the null hypothesis. Given no human-to-human transmission, all the observed case-patients must have been infected by the zoonotic source. Since the exposure to the zoonotic source is assumed constant for each person on each day, the null likelihood function will not change if we reassign the infection and symptom status of the observed case-patients to a different group of people in the population. By performing such reassignment many times, we obtained a collection of datasets that were each equally likely to have been observed had there been no human-to-human transmission. The values of the likelihood ratio statistic calculated from these datasets form the null distribution for statistical testing. This method is referred to as a permutation test. The p value is given by the proportion of the reference values that are equal to or larger than the observed likelihood ratio statistic value. More technical details are given in the online Technical Appendix.

The probability of infection by the zoonotic source may not be estimable together with SAR_1 or SAR_2 from an observed cluster. In such a situation, a statistical test of the occurrence of human-to-human transmission is still meaningful because the likelihood ratio test statistic is still estimable from the permuted datasets.

Data Required

A list of the inputs that are required for estimation and statistical testing are listed in the Table. Three categories of input parameters are required for this estimation model: outbreak-wide, individual level, and analysis parameters. The duration of the outbreak, the duration of the incubation period for the pathogen, and the minimum and maximum durations of the infectious period for the pathogen are the required outbreak-wide inputs. For each person, their residential location (neighborhood and household), their de-

Table. Parameters and data used in analysis

Category	Parameter/data	Required*
Entire outbreak	Outbreak begin date	X
	Outbreak end date	X
	Latent/incubation period, d†	X
	Infectious period, d†	X
All persons	Neighborhood of residence	X
	Household of residence	X
	Sex	X
	Age, y	X
	Case status (yes or no)	X
Case-patients	Whether outbreak index case-patient (yes or no)	X
	Date of illness onset	X
	Outcome (recovered, died, or don't know/still ill)	X
	Date of outcome	X
	Dates of hospitalization	O
	Period of receiving treatment (dates)	O
Non–case-patients	Dates of hospitalization	O
	Period of prophylactic treatment (dates)	O
Inter-residence visits	Identifier for visiting person	X
	Neighborhood visited	X
	Household visited	X
	Dates of the visit	X
Analysis parameters	End of exposure to the common source of infection (date)	X
	Final day of observation (date)	X
R_0 estimation	Mean no. residents per household	X‡
	Mean no. community contacts per person/d	X‡

*X, required; O, optional; R_0, basic reproduction number.
†The user defines the distribution of this period, including the minimum and maximum length of the period.
‡Required to estimate R_0.

mographic characteristics (sex and age), and whether they were a case-patient or not are required input parameters. Case-patients require additional input of their illness-onset dates, types of outcome, outcome dates, and whether or not they are the index patient in the outbreak. Hospitalization and treatment dates (considered prophylactic for nonpatients) are optional input parameters for each person. For each person who visits another residence during the outbreak period, his or her identifiers, the neighborhood and household visited, and the start and end dates of the visit are required inputs. Analysis-related inputs include the last date of community exposure to potential common sources of infection, the last date of observation, and inputs for R_0 estimation (mean number of residents per household and mean number of out-of-residence contacts per person per day). An expanded version of the model will require the input of other exposure information such as from schools or hospitals.

Results

For the outbreak in Indonesia, online Appendix Figure 1 shows that the incubation period had a probable range of 3–7 days and the infectious period, a probable range of 5–13 days. Thus, we let the incubation period have a uniform distribution of 3–7 days (mean 5 days) and the infectious period a uniform distribution of 5–13 days (mean 9 days). For the data shown in online Appendix Figure 1, only the household SAR (SAR_1) can be estimated. We determine that human-to-human spread did occur by rejecting the null hypothesis of no human-to-human transmission ($p = 0.009$). The estimated household SAR is 0.29 (95% confidence interval [CI] 0.15–0.51). Thus, a single infected person in a household infected another household member with the probability of 0.29. The average household size for rural Indonesia is ≈5 people. Because we do not have an estimate of the community SAR, we have an estimate of the lower limit of the local R_0, i.e., 1.14 with a 95% CI of 0.61–2.14. A sensitivity analysis on the distribution of the incubation and infectious period shows that the test and estimates for SAR_1 and R_0 are insensitive to uncertainty about these distributions within plausible ranges.

For the outbreak in Turkey, all the parameters are estimable, but we do not reject the null hypothesis of no human-to-human transmission ($p = 0.114$). Our estimate of the daily probability of infection from the common source is 0.011 (95% CI 0.005–0.025).

Discussion

We have presented statistical evidence that the strain of HPAI (H5N1) that caused the family cluster of human cases in northern Sumatra was spread from human to human and that the household SAR was 29%. This household

SAR is similar to statistical estimates for interpandemic influenza A in the United States (12.7%–30.6%) (*18,19*). The mean incubation period of this strain appears to have been ≈5 days, nearly twice as long as for past pandemic strains and current interpandemic strains of influenza. The CI for the estimated lower bound for the local R_0 covers 1. Therefore, even though we determined that human-to-human transmission probably occurred, whether the virus was capable of sustained human-to-human transmission is not clear. This virus may have required very close human contact to be transmitted. Even with no intervention, the finding that $R_0 = 1.14$ indicates that the chance that a single introduction would result in any further spread is ≈12%. In addition, the reported prophylactic use of oseltamivir may have played some role in limiting further spread. We did not find statistical evidence of human-to-human spread for the outbreak in eastern Turkey. This does not mean that no low-level human-to-human spread occurred in this outbreak, only that we lack statistical evidence of such spread. The power would be too low to detect such spread for an outbreak with 7 total cases and small SARs (*17*).

We did not consider the role of heterogeneity—such as age, sex, treatment status, or quarantine—in transmission. The parameters could be made to be functions of time-dependent covariates, as we have done with similar models (*16,19,20*). We can easily extend the model used here for covariates; however, we must have sufficient data to support such models.

Computer simulations have shown that the targeted use of influenza antiviral agents could be effective in containing a potential pandemic strain of influenza at the source (*21,22*), if initiated within 3 weeks of the initial case in the community, and if the R_0 is <1.8. This strategy, known as targeted antiviral prophylaxis, involves treating identified index patients in a mixing group and offering a single course of prophylaxis to the contacts of these index patients in predefined close contact groups, i.e., households at a minimum but also possibly neighborhood clusters, preschool groups, schools, and workplaces. In addition, the voluntary household quarantine of suspected close contacts of case-patients was recommended. Targeted antiviral prophylaxis at the household and neighborhood cluster level was carried out for the outbreak in Sumatra.

Ascertaining whether a potential pandemic strain of influenza is capable of sustained human-to-human transmission and estimating key transmission parameters are important. To estimate more than the household SAR, more detailed community data need to be collected. This would include a complete census of potentially exposed households and persons in the area where immediate transmission could occur from both potential zoonotic and human sources. Such data would enable estimation of important parameters and a more complete estimate of the R_0 rather than just the lower limit.

We have developed a software application, TRANSTAT, for implementing these analyses. This application provides a stand-alone environment for the entry, storage, and analysis of data from outbreaks of acute infectious diseases. A partial list of the input information is given in the Table. The statistical methods presented here can be applied to the data along with several standard epidemiologic tools. This information system would allow for real-time analysis and evaluation of control measures for an outbreak. We would encourage outbreak investigators to use this tool, taking care to input data on the exposed nonpatients as well as case-patients. The authors will provide a link to this software upon request.

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Dr Yang is a staff scientist in the Biostatistics and Biomathematics Program in the Division of Public Health Sciences at the Fred Hutchinson Cancer Research Center, Seattle, Washington, USA. His primary research interest is in the statistical and mathematical analysis of infectious disease data and intervention studies.

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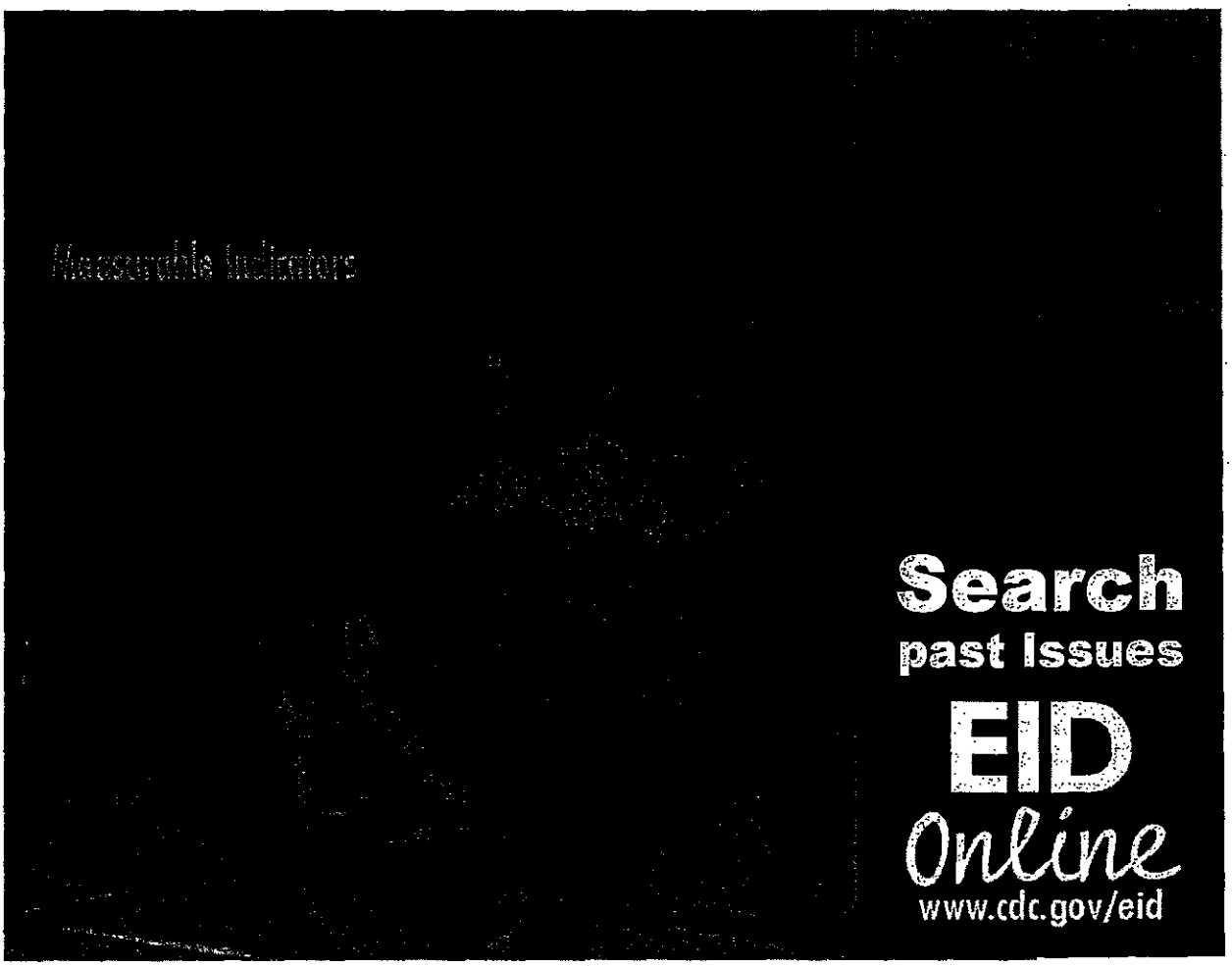
Address for correspondence: Ira M. Longini, Jr, Program in Biostatistics and Biomathematics, Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA; email: longini@scharp.org



別紙様式第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 平成 19 年 10 月 12 日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ人血清ｱﾙﾌﾞﾐﾝ (^{99m}Tc)		研究報告の公表状況	Jiang Gu, et al. H5N1 infection of the respiratory tract and beyond: a molecular pathology study The Lancet Vol. 370, 29 September 2007, P 1137 -45	公表国 中国	
販売名（企業名）	テクネアルブミンキット（富士フイルムＲＩファーマ株式会社）					

研究報告の概要

背景： トリインフルエンザ H5N1 のヒトへの感染は、呼吸器系の症状と高い致死率を特徴とした新興感染症である。これまでの研究ではトリインフルエンザ H5N1 のヒトへの感染は、肺とは異なる臓器もターゲットにしている事を示している。

方法： H5N1 インフルエンザウイルスに感染していた 2 人の成人(一人は男性、一人は妊娠している女性)の死後の組織と、その女性の胎児について調べた。In-situ Hybridization(血球凝集素と核蛋白のセンス、アンチセンスプローブ)と免疫組織化学染色（血球凝集素と核蛋白に対するモノクローナル抗体を用いた）が選択された組織で行なわれた。逆転写酵素(RT)PCR、リアルタイム RT-PCR、Strand-Specific RT-PCR、および塩基配列に基づく増幅(NASBA)検出法が臓器組織サンプルのウイルスの RNA を検出するために実施された。

結果： 肺の II 型上皮細胞、気管の繊毛・非繊毛上皮細胞、リンパ節の T 細胞、脳の神経細胞、胎盤のホフバウアー細胞と細胞栄養芽層でウイルスの遺伝子配列と抗原を検出した。ウイルスの遺伝子配列（ウイルス抗原は検知されなかったが）は腸管粘膜で検出された。胎児では、肺、循環する単核細胞、肝臓のマクロファージで、ウイルスの塩基配列と抗原を検出した。臓器と胎児中のウイルスの塩基配列の存在は RT-PCR, Strand-specific PCR、real-time PCR 及び NASBA でも確認された。

注釈：肺に加え、H5N1 インフルエンザウイルスは気管に感染し、脳を含む他の臓器に拡散する。又、ウイルスは胎盤を通じて母親から胎児へ感染する。

報告企業の意見	今後の対応
高病原性トリインフルエンザ（H5N1）が「胎盤を通じて母親から胎児へ感染した」ことを確認した初めての報告である。本報告では In-situ Hybrydization 法、免疫組織化学染色法、逆転写酵素 PCR 法等の手法によって、胎盤の絨毛膜中に多数の感染細胞が検出され、胎児の肺・循環する単核細胞・肝臓のマクロファージにウイルスの塩基配列の存在を確認したとあり、本報告は新たに判明した感染経路に関するもの且つ重大な感染症に関するものと判断する。	本報告はヒト血液を原料とする血漿分画製剤とは直接関連するものではなく、現時点で特に当該生物由来製品に関し、措置等を行う必要はないと判断する。血清疫学研究ではこれまでにヒトインフルエンザの経胎盤感染の証拠は示されておらず、トリインフルエンザの方が子宮や胎児に容易に到達する可能性を示唆していることから、今後も同様の情報収集が必要であると考える。

使用上の注意記載状況・その他参考事項等

特になし

MedDRA Version(10.1)

DRL 2007-003

H5N1 infection of the respiratory tract and beyond: a molecular pathology study

Jiang Gu, Zhigang Xie,* Zhancheng Gao,* Jinhua Liu,* Christine Korteweg,* Juxiang Ye, Lok Ting Lau, Jie Lu, Zifen Gao, Bo Zhang, Michael A McNutt, Min Lu, Virginia M Anderson, Encong Gong, Albert Cheung Hoi Yu, W Ian Lipkin*

Summary

Background Human infection with avian influenza H5N1 is an emerging infectious disease characterised by respiratory symptoms and a high fatality rate. Previous studies have shown that the human infection with avian influenza H5N1 could also target organs apart from the lungs.

Methods We studied post-mortem tissues of two adults (one man and one pregnant woman) infected with H5N1 influenza virus, and a fetus carried by the woman. In-situ hybridisation (with sense and antisense probes to haemagglutinin and nucleoprotein) and immunohistochemistry (with monoclonal antibodies to haemagglutinin and nucleoprotein) were done on selected tissues. Reverse-transcriptase (RT) PCR, real-time RT-PCR, strand-specific RT-PCR, and nucleic acid sequence-based amplification (NASBA) detection assays were also undertaken to detect viral RNA in organ tissue samples.

Findings We detected viral genomic sequences and antigens in type II epithelial cells of the lungs, ciliated and non-ciliated epithelial cells of the trachea, T cells of the lymph node, neurons of the brain, and Hofbauer cells and cytotrophoblasts of the placenta. Viral genomic sequences (but no viral antigens) were detected in the intestinal mucosa. In the fetus, we found viral sequences and antigens in the lungs, circulating mononuclear cells, and macrophages of the liver. The presence of viral sequences in the organs and the fetus was also confirmed by RT-PCR, strand-specific RT-PCR, real-time RT-PCR, and NASBA.

Interpretation In addition to the lungs, H5N1 influenza virus infects the trachea and disseminates to other organs including the brain. The virus could also be transmitted from mother to fetus across the placenta.

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*Contributed equally to the study as first authors

Infectious Disease Centre (Prof J Gu MD, L T Lau PhD, J Lu PhD, Prof E Gong MD, Prof A C H Yu PhD), Department of Pathology, School of Basic Medical Sciences (Prof J Gu, Z Xie MD, C Korteweg MD, J Ye BS, Prof Z Gao MD, Prof B Zhang MD, Prof M A McNutt MD, M Lu MD, V M Anderson MD, Prof E Gong), People's Hospital (Prof Zh Gao MD), Neuroscience Research Institute (Prof A C H Yu, L T Lau, J Lu), Peking University, Beijing, China; College of Veterinary Medicine, China Agricultural University, Beijing, China (Prof J Liu PhD); Department of Pathology, State University of New York, Health Science Center at Brooklyn, New York, NY, USA (Prof J Gu, V M Anderson); Mailman School of Public Health, Columbia University, New York, NY, USA (Prof W I Lipkin MD)

Correspondence to:
Prof Jiang Gu, Infectious Disease Centre, Peking University, Beijing, 100083 China
jianggu@bjmu.edu.cn

Introduction

A pandemic outbreak of human infection with avian influenza H5N1 currently poses a potentially serious health threat worldwide. Since the outbreak of infection with avian influenza H5N1 virus in 2003, WHO has reported 277 laboratory confirmed cases in ten countries with a mortality rate of about 60%.[1] So far the virus has spread only from animals to human beings. However, human-to-human transmission potentiated by viral genomic mutation and reassortment of genomic subunits could be imminent. Recently, the first cases of probable human-to-human transmission have been reported.[2,3] The H5N1 influenza A virus is a negative-stranded RNA virus in which the genome consists of eight segments encoding ten viral proteins including haemagglutinin, neuraminidase, polymerase proteins, and nucleoprotein.[4]

Little is known about the specific effects in organs and cells targeted by the virus. The infection initially seemed to be restricted to the lungs, but later reports[5,6] have suggested that influenza A H5N1 could disseminate beyond the lungs. For various reasons (eg, religion), full autopsies of H5N1-infected human cases can often not be obtained. Accordingly, only a few reports[2,5,7–9] have described histopathology and virus distribution in H5N1 cases. Studies using in-situ hybridisation to detect viral genomic sequences in target cells have not been reported thus far.

We present clinicopathological data from H5N1 autopsies of two unrelated Chinese cases, as well as the histopathological changes and pattern of infection in the placenta and fetus from one of the patients, who was pregnant at the time of death. To gain further insight into the tissue tropism of influenza A H5N1 virus, we used in-situ hybridisation and immunohistochemistry to analyse viral localisation in various organs. Reverse transcription (RT) PCR, real-time RT-PCR, and nucleic acid sequence-based amplification (NASBA) H5 detection assays were also done to detect viral RNA in tissue samples, as well as strand-specific RT-PCR.

Methods

Patients

The clinical data of patient 1 have previously been published in detail.[10] A 24-year-old Chinese woman from China's Anhui province who was 4 months pregnant presented with a 6-day history of fever, cough, and dyspnoea. 2 weeks before admission, she had handled birds, several of which had died. On admission, she was lymphopenic, confused, and irritable, had bilateral infiltration on chest radiograph, and substantially reduced oxygen saturation. She was placed on a ventilator and treated with antibiotics, corticosteroids (hydrocortisone 400 mg on day 6 and day 7, and methylprednisolone 160 mg on day 8 and 240 mg on day 9), and fluids, but died 62 h after admission, 9 days after the onset of symptoms. No antiviral treatment was given.

See Online for webtable

Patient 2 was a 35-year-old Chinese man from China's Jiangxi province who had a 6-day history of fever and productive cough. He had participated in selling birds, of which several had died. On admission, chest radiographs showed evidence of pneumonia, and laboratory results showed abnormally increased hepatic-associated and cardiac-associated enzymes and lymphopenia. He was treated with antibiotics. Corticosteroids were given initially with methylprednisolone 40 mg on day 10 and then 120 mg per day for 17 days. Antiviral treatment was given, including rimantadine 100 mg twice daily on days 10 and 11 and then oseltamivir 150 mg per day from day 11 for 10 days. After admission, he became increasingly irritable and confused, followed by lowered consciousness. He developed respiratory failure and mechanical ventilation was initiated. In a sputum culture, several gram-positive microorganisms and *Candida albicans* were isolated. Antifungal drugs were added to his treatment. He developed multiple organ failure and died 27 days after the onset of symptoms.

The Chinese Centre for Disease Control and Prevention confirmed human infection with avian influenza H5N1 in both patients. RT-PCR detected H5N1 viral sequences in nasal swabs and nasopharyngeal aspirates, which were obtained on day 6 of illness for patient 1 and day 10 for patient 2. Viruses were isolated from the nasopharyngeal aspirate cultures, and designated as influenza A/Anhui/1/2005 virus[9] in patient 1 and A/Jiangxi/1/2005 virus in patient 2. The haemagglutinin genes of viruses in patient 1 (GenBank accession number DQ371928)[10]

See Online for webappendix

and patient 2 (webappendix)[11] were sequenced. The receptor-binding sites of both viruses were identical to those of previous H5N1 isolates.[12] H5N1 viruses isolated from both patients were susceptible to both the M2 inhibitors amantadine and rimantadine, and the neuraminidase inhibitors oseltamivir and zanamivir.

Both cadavers were stored at 4°C and underwent autopsy about 18–20 h after death. The autopsies were done following conventional protocols and strict safety procedures.[13] Tissue samples from all major organs and tissues were taken and fixed in 4% formalin. The brain of patient 1 was not available for investigation.

Immunohistochemistry

Immunohistochemistry was done on the basis of the technique of Lin and colleagues, with antigen retrieval by a standard technique.[14,15] To detect viral antigen, tissue slides of 4 μm thickness were incubated with mouse monoclonal antibodies to nucleoproteins and haemagglutinin. Furthermore, monoclonal antibodies to the following cell markers were used: CD68 (for macrophages), CD3 (T lymphocytes), CD20 (B lymphocytes), CD8 (cytotoxic T cells), S100 (dendritic cells), cytokeratin AE1/AE3 (epithelial cells), surfactant protein A (type II pneumoncytes), tubulin-β (ciliated epithelial cells), placental alkaline phosphatase (PLAP, syncytiotrophoblasts), E-cadherin (cytotrophoblasts),[16] neurofilament (neurons), neuron-specific enolase (neurons), and factor VIII (endothelial cells, webtable).[17] For controls, we used unrelated antibodies in place of the primary antibody.

In-situ hybridisation

For the development of probes, we used haemagglutinin (GenBank accession number DQ100556) and nucleoprotein gene sequences (DQ100560) of the H5N1 A/black-headed gull/Qinghai/1/2005 virus, which was recently isolated from a migratory bird at China's Qinghai lake.[18] Plasmids were generated by cloning of the full haemagglutinin gene (1779 bp) and full nucleoprotein gene (1565 bp) into a plasmid vector PGEM-T (Promega, Madison, WI, USA) yielding pGEM-HA for haemagglutinin and pGEM-NP for nucleoprotein. Both plasmids were linearised with appropriate restriction enzymes. Two sense and two antisense RNA probes were prepared by in-vitro transcription with T7 and Sp6 RNA polymerase (Promega) in the presence of digoxigenin-UTP (Roche Diagnostics, Penzberg, Germany). Since H5N1 is a negative-stranded RNA virus, sense probes were defined as the probes that detect the viral RNA (negative-stranded), whereas antisense probes detected mRNA and complement RNA (cRNA), which are both positive-stranded.

Briefly, before hybridisation, all solutions were prepared with diethyl pyrocarbonate (DEPC)-treated water.[19] After deparaffinisation and rehydration, tissue sections of 4 μm thickness were treated with proteinase K digestion or microwave heating. Tissue sections were then incubated with a hybridisation cocktail containing 50 μg/mL of one of the four sense and antisense probes at 45°C for 16 h. All sense and antisense probes were applied separately on consecutive tissue sections. After blocking with horse serum (1:100), sections were incubated with alkaline phosphatase-labelled digoxigenin antibody (1:500, Roche Diagnostics, Penzberg, Germany) for 1 h, and the reaction products were colourised with nitroblue tetrazolium/5-bromo-4-choloro-3-indolyl phosphate (Promega).

As a positive control, we used brain tissue samples of a black-headed gull, for which H5N1 infection of the brain was confirmed by viral isolation.[18] We used lung tissues from a mouse infected with H9N2 influenza virus as a negative control. Negative controls also included an unrelated antisense probe against the fragment of the polymerase gene (*R1AB*) of the severe acute respiratory syndrome-associated coronavirus (SARS-CoV),[20] as well as H5N1 in-situ hybridisation probes to tissues (including lung and tracheal) obtained from seven adults who died from infectious lung diseases other than H5N1 influenza (four, SARS; one, purulent bronchitis; two, pneumonia), one adult who died from a non-infectious disease (gastric ulcer), one pregnant woman who died from an amniotic embolism, and one aborted fetus.

We identified the cell types infected by the virus with double labelling by combining in-situ hybridisation for

viral genomic sequences and immunohistochemistry for one of the cell-associated markers. To identify placental cells and cerebral neurons containing viral sequences or antigens, consecutive sections adjacent to the sections used for in-situ hybridisation or immunohistochemistry were immunostained with CD68, E-cadherin, PLAP, neurofilament, or neuron-specific enolase. Tissues from the pharynx, nose and paranasal sinuses, and lymph nodes other than hilar nodes were not available for investigation.

On preliminary investigation of lung tissues, we noted a contrast between the extent of histological damage and the limited number of positive pneumocytes (patient 1) or absence of positive pneumocytes (patient 2) shown by in-situ hybridisation and immunohistochemistry studies. Therefore, we did extensive pulmonary sampling for further histological assessment to ensure that the results were representative.

Expected localisation of signals from in-situ hybridisation and immunohistochemistry

After receptor-mediated endocytosis of the virus, the polymerase proteins, nucleoprotein, and encapsidated RNA segments migrate to the nucleus of the infected cell. In the nucleus, RNA segments are transcribed into mRNA and cRNA. mRNA is subsequently translated to viral proteins (eg, haemagglutinin and nucleoprotein) in the cytoplasm. Newly synthesised nucleoprotein is transported back to the nucleus. cRNAs function as antigenomic templates for the production of progeny RNA segments in the nucleus. Assembly of progeny gene segments and proteins occurs in the cytoplasm.[4]

Therefore, sense and antisense signals after in-situ hybridisation for nucleoprotein and haemagglutinin could be seen in both the nucleus and cytoplasm of infected cells, as well as immunohistochemical signals for nucleoprotein in the nucleus and for haemagglutinin in the cytoplasm. Since antisense probes hybridise to mRNA and cRNA, a positive signal would probably indicate active viral replication.

Bronchi	-/-	-/-	-/-	-/-	+/+	+/+
Lymph nodes	+/+‡	–	+/+‡	+/+‡	–	–
Heart	-/-	-/-	-/-	-/-	-/-	-/-
Hepatocytes	-/-	-/-	-/-	-/-	-/-	–
Kidneys	-/-	-/-	-/-	-/-	-/-	–
Brain	–	–	+/+	+/+	–	–
Cytotrophoblasts	+/+	+/+	n/a	n/a	n/a	n/a
Circulating mononuclear cells	-/-	-/-	-/-	+/+	+/+	+/+

Table 1: Results of in-situ hybridisation and immunohistochemistry in selected organs and cell types

RT-PCR, real-time RT-PCR, and NASBA

RNA was extracted from deparaffinised tissue samples, or directly extracted from formalin-fixed tissues after overnight incubation with proteinase K (10 µg/µL, Ameresco, Cleveland, OH, USA). We did RNA extraction with Trizol (Invitrogen, CA, USA), and PCR as previously described.[21] The haemagglutinin gene of the H5N1 virus was detected with H5for as the forward primer and H5rev as the reverse primer for H5 gene amplification (panel). Reamplification was done for specific samples with the same set of primers if no band was seen in gel electrophoresis. We obtained negative controls for RT-PCR from uninfected tissues (eg, lung, brain, placenta, and intestine) from three patients who died from non-infectious diseases. RT-PCR for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was assessed in parallel as an internal control.

To detect viral RNA in fetal tissues, real-time RT-PCR (H5 avian influenza virus Nucleic Acid Amplification Fluorescent Quantitative Detection Kit, PG Biotech, Shenzhen, China) was used as recommended by the manufacturer and the national standards of the People's Republic of China.

Panel: Nucleotide sequences of primers used in RT-PCR and sequencing, RT-PCR, and NASBA

H5for
5'-ATTGAYAAYGAAGTGAATGGAAA-3'

H5rev
5'-CCATAAAGAYAYACCAGCTA-3'

H5for
5'-CTCAAGTTGGTCAGTAAGTGAYAATGGAATTATAAATGCAA-3'

tag-H5rev
5'-AGTTAGTGGTCAGTACCATAAAGAYACCAGCTA-3'

GAPDH
5'-CTCAGTGTAGCCATGCAA-3'

NASBA capture probe
5'-TCTGCTAGTTGCTACCATGCTGAAT-3'

NASBA detection probe
Biotin-GATGCAAGCTCGCATATCAG

Figure: Nucleotide sequences of primers used in RT-PCR and sequencing, RT-PCR, and NASBA

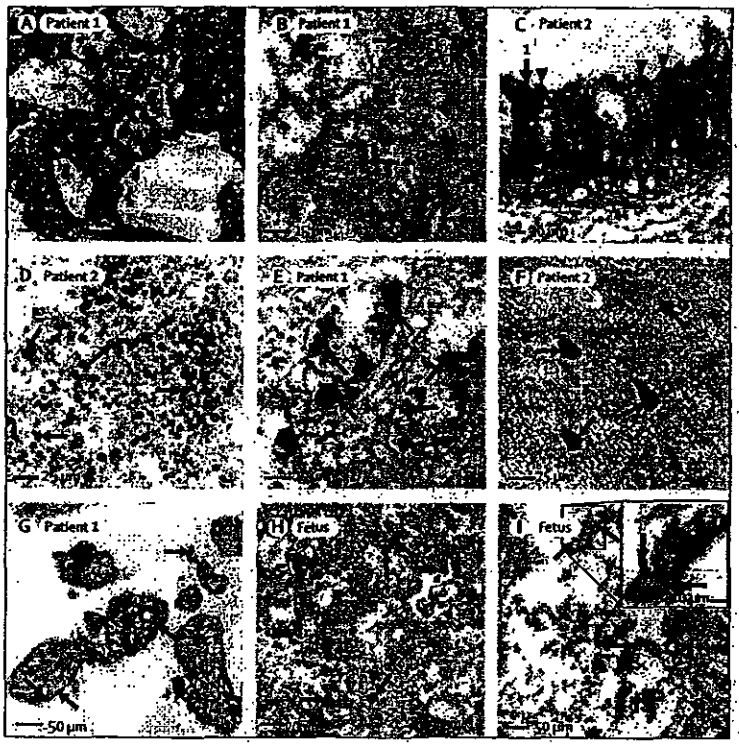

Figure 1: In-situ hybridisation locating gene sequences of H5N1 viral antigens (nucleoprotein and haemagglutinin)

Signals seen with nitroblue tetrazolium/5-bromo-4-choloro-3-indolyl phosphate (purple-blue) and immunohistochemical signals with 3-amino-9-ethylcarbazole (double labelling, brown-red). No counterstaining done unless stated otherwise. (A) Lung tissue showing severe damage, hyaline membrane formation, and oedema (by haematoxylin and eosin staining), by contrast with limited number of cells positive for in-situ hybridisation in lung tissue of patient 1 (figure 1B). (B) Positive signals (with nucleoprotein sense probe) in nuclei of isolated pneumocytes (arrows). (C) Double labelling of in-situ hybridisation (with nucleoprotein antisense probe) and immunohistochemistry (with tubulin-β antibody; brown, arrowheads) showing dark-blue viral genomic sequences (arrows) in cytoplasm of tubulin-negative non-ciliated cell (arrow 1) and tubulin-positive ciliated cell (arrow 2) in trachea. No signal seen in nuclei (lightly counterstained with methyl-green). (D) Positive signals (with nucleoprotein sense probe) in several mononuclear cells in lymph node (arrows, lightly counterstained with methyl-green). (E) Positive signals (with nucleoprotein antisense probe) in mucosal epithelial cells of small intestine (arrows, lightly counterstained with methyl-green). (F) Positive signals (with nucleoprotein sense probe) in brain cells (arrows) from left parietal lobe (lightly counterstained with methyl-green), mainly located in cytoplasm and confirmed to be neurons from immunostaining for neurofilament or neuron-specific enolase (webfigures 2O and 2P). (G) Positive signals (with nucleoprotein antisense probe) in large mononuclear cells with morphological features of cytotrophoblasts in periphery of chorionic villus (arrows). Cells confirmed as cytotrophoblasts (webfigure 4D). No positive signals noted in any syncytiotrophoblasts. (H) Positive signals (with nucleoprotein sense probe) in fetal liver cells (arrows), confirmed as Kupffer cells (webfigure 1F). (I) Positive signals (with haemagglutinin sense probe) in bronchiolar epithelial cells in fetal lung tissue (arrows).

Strand-specific RT-PCR was undertaken on the basis of the technique of Yue and co-workers,[22] with minor modification. Briefly, two-part reactions were used. First, the RT reaction was done in the presence of tagged primer, tag-H5for or tag-H5rev (panel). A third of cDNA products then underwent PCR with primers Tag/H5rev or Tag/H5for. We reamplified under the same PCR conditions for a specific sample if no band was seen after gel analysis of the first PCR reaction.

See Online for webfigure 1

Virus-specific RNA was also detected with the avian influenza virus (H5 subtype) NASBA diagnostic test kit (MP version, Hong Kong DNA Chips, Hong Kong). The test was done as previously described[23] with H5-specific capture and detection probes (panel). Absorbance of the amplified product was measured at 405 nm by an ELISA plate reader (Bio-Rad, Hercules, CA, USA). A negative control from the test kit was also included for H5 detection. The study was approved by the internal review board and ethics committee of the Peking University Health Science Centre.

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

The microscopic features of both patients were similar, apart from more extensive fibroproliferative changes in the lungs of patient 2. Both lungs of patient 1 showed features of diffuse alveolar damage (figure 1A) and focal desquamation of epithelial cells into alveolar spaces without evidence of type II pneumocyte hyperplasia. In the lungs of patient 2, patchy foci of consolidated bronchopneumonia and areas of fibrosis were seen. We found variable numbers of macrophages in the alveoli (especially in patient 2), and moderate numbers of scattered neutrophils and rare lymphocytes in the interstitial spaces. Both patients had substantially depleted lymphoid tissue in the spleen, lymph nodes, and mucosal lymphoid tissue in the gastrointestinal tract. The liver in both patients had spotty necrosis. In both patients, we detected very low numbers of macrophages with haemophagocytosis in the spleen, lymph nodes, and liver. The kidneys showed extensive tubular necrosis. Other organ systems showed no pronounced histological changes, apart from hypertrophy in the thymus of patient 2.

The placenta showed development appropriate for the length of gestation. We saw scattered foci of syncytiotrophoblast necrosis, sometimes with associated dystrophic calcification. Whether this finding was induced virally or was the sole result of maternal shock is unclear. Acute necrotising deciduitis was detected focally, and regarded as consistent with maternal shock.

Fetal tissues mostly showed no specific histopathological findings, and development was also consistent with gestational age. However, sections of fetal lung showed oedema and very small numbers of scattered interstitial neutrophils, which raised the possibility of mild acute interstitial pneumonitis, although this appearance was notably less severe than that seen in patients 1 and 2 (webfigure 1).

Sense and antisense probes for in-situ hybridisation detected viral genomes focally in tissue samples from various organs (table 1). The two sets of probes (for

haemagglutinin and nucleoprotein) generated identical staining results. In both autopsies, the trachea, small intestines, and lymph nodes showed positive signals of in-situ hybridisation, although pneumocytes were positive only in patient 1. In the lungs, sense probes hybridised in the nuclei of pneumocytes (figure 1B), whereas antisense probes hybridised in both the cytoplasm and nuclei. In all other organs with positive signals, both sense and antisense were present mainly in the cytoplasm of infected cells.

In the respiratory tract, we detected positive signals in tracheal epithelial cells and alveolar epithelial cells (figures 1B and 1C, webfigure 1). However, only an estimated 10–20% of epithelial cells in the trachea and about 5% of epithelial cells in the alveoli showed positive signals. Both bronchi and bronchioles were negative. Double labelling combining in-situ hybridisation and immunohistochemistry with tubulin-β antibody showed that both ciliated (tubulin-β-postive) cells and non-ciliated (tubulin-β-negative) cells of the trachea had viral sequences (figure 1C, webfigure 2). We also found putative basal cells to be infected. Double labelling with antibodies for cytokeratin and surfactant protein A showed that the positive alveolar cells were type II pneumocytes (webfigures 1C and 1D). In-situ hybridisation showed no positive staining in endothelial cells, macrophages, lymphocytes, fibroblasts, or any other cell type in the lungs or blood. However, we found positive viral signals in the cytoplasm of mononuclear cells in hilar lymph nodes (figure 1D). Double labelling and consecutive sections showed that cells positive for in-situ hybridisation were T lymphocytes (ie, positive for CD3 and negative for CD68, CD20, and S100, webfigure 3). Additionally, positive signals of intracytoplasmic viral sequences were present in mucosal epithelial cells of the small intestine (>50% in some intestinal segments, figure 1E). We also detected viral sequences in the cytoplasm (and to a much lesser extent in the nuclei) of brain cells from patient 2 (figure 1F, webfigure 1). Double labelling with neural markers neurofilament or neuron-specific enolase showed that these H5N1-positive cells were neurons (webfigures 1O and 1P). Table 2 shows the topographic distribution of cells with positive signals from in-situ hybridisation. No positive signals were seen in the heart, liver, spleen, kidneys, oesophagus, bone marrow, or stomach.

Placental tissue samples showed a large number of infected cells in chorionic villi (webfigure 4). Most positive cells were localised in the connective tissue core of these villi. These cells were confirmed (by labelling with monoclonal antibodies to CD68 and PLAP in consecutive sections) to be Hofbauer cells but not syncytiotrophoblasts. A subgroup of the cells with positive signals was found in the periphery of chorionic villi (figure 1G), which morphologically resembled cytotrophoblastic cells and were confirmed by immunostaining with E-cadherin antibody on consecutive sections. No positive signal was seen in syncytiotrophoblasts.

In the fetus, in-situ hybridisation identified viral sequences in the lungs, circulating mononuclear cells (webfigure 1), and mononuclear cells in the liver (figure 1H). The latter cells were identified as macrophages (Kupffer cells) by double labelling with antibody to CD68 (webfigure 1). Both sense and antisense probes were

See Online for webfigures 2, 3, and 4

	ISH		RT-PCR	NASBA (400 nm)
	Antisense	Sense	Positive/Total	Positive
Frontal lobe	–	–	+ 2·499	+
Occipital lobe	+	+	+ 2·312	+
Temporal lobe	+	+	+ 2·466	+
Thalamus	+	+	+ 2·295	+
Pons	+	+	+ 2·031	+

Table 2: Results of in-situ hybridisation, RT-PCR, and NASBA for detection assay in selected brain regions of patient 2

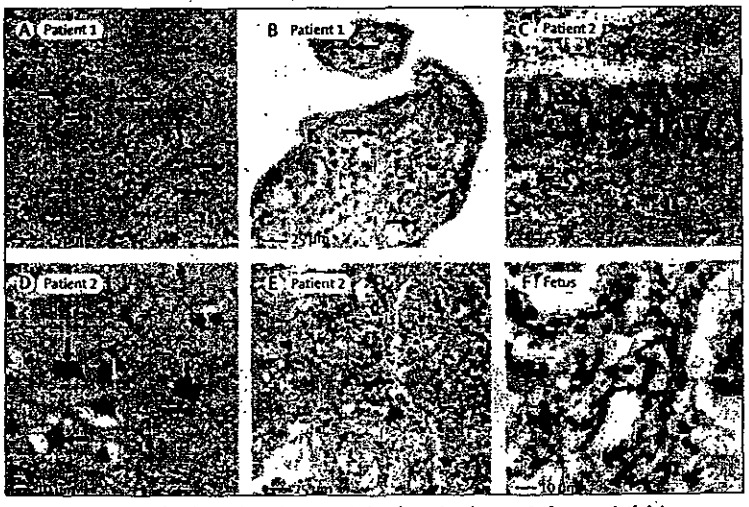

Figure 2: Immunohistochemical staining for H5N1 viral antigens (nucleoprotein, haemagglutinin)
Positive signals seen with diaminobenzidine (brown; Zymed Laboratories, San Francisco, CA, USA) or 3-amino-9-ethylcarbazole (red-brown; Sigma, St Louis, MO, USA). Slides counterstained with haematoxylin. (A) Positive staining (with nucleoprotein antibody) in nuclei and cytoplasm of pneumocytes (arrows). (B) Positive signals (with nucleoprotein antibody) in nuclei of mononuclear cells (arrows) with morphological features of macrophages in core of chorionic villus. Immunostaining in adjacent sections indicate cells to be Hofbauer cells (webfigure 4). (C) Positive signals (with nucleoprotein antibody) in nuclei of epithelial cells of trachea (arrows). (D) Positive staining (with nucleoprotein antibody) in cytoplasm and nuclei of neurons (arrows) from hippocampus. (E) Positive staining (with haemagglutinin antibody) in mononuclear cell in lymph node (arrow). (F) Positive staining (with haemagglutin antibody) in cytoplasm of pneumocytes (arrows) in fetal lung tissue.

positive, mainly in the nuclei of pneumocytes and in a few detached epithelial cells of the bronchi (figure 1I). In Kupffer cells and circulating mononuclear cells, sense and antisense probes showed positive signals in both the cytoplasm and nucleus. The fetal lungs appeared to have more positively stained cells than did the maternal lungs.

The specificity of in-situ hybridisation was established by the results with the negative and positive controls (webfigure 1). The brain sections of the black-headed gull showed almost all neurons with positive signals for in-situ hybridisation, indicating the high detecting sensitivity of this technique.

See Online for webfigure 5

Distribution of immunohistochemical staining (table 1) was consistent with that of in-situ hybridisation, apart from the absence of viral antigens in the intestines. Positive staining for nucleoprotein and haemagglutinin was detected in pneumocytes (figure 2A) and cytotrophoblasts and Hofbauer cells in the placenta (figure 2B) in patient 1; as well as in tracheal epithelial cells (figure 2C), the brain (figure 2D), and T lymphocytes in hilar lymph-node tissue (figure 2E) in patient 2. The fetus showed positive staining in bronchial epithelial cells, pneumocytes (figure 2F), and circulating mononuclear cells. Nucleoprotein was mainly detected in the nucleus and haemagglutinin in the cytoplasm. Negative controls validated the specificity of the immunohistochemistry protocol (webfigure 5).

All organs tested showed positive RT-PCR results, apart from the lymph nodes of patient 2 (tables 2 and 3, figure 3). All non-paraffin-embedded samples were positive for H5 expression. However, paraffin-embedded tissues only showed H5 expression after reamplification of the RT-PCR products. NASBA showed positive results on both types of samples without the need of reamplification.

With real-time RT-PCR, viral RNA was detected in the lungs and liver of the fetus (table 3). GAPDH was an internal control for successful RNA extraction in these assays. Positive-stranded RNA was detected in the heart and placenta of patient 1 and in the lungs, trachea, intestines, and brain of patient 2 (table 3, figure 3). The specificities of the RT-PCR and NASBA were further confirmed by use of the negative controls.

Trachea	–	–	–	Y	Y	Y	–	–	n/a	1·236	Y
Brain	–	–	–	Y	Y	Y	–	–	n/a	2·424	Y
Spleen	Y	Y	N	Y	N	N	–	1·326	Y	1·442	Y
Kidneys	Y	Y	N	Y	N	N	N	2·371	Y	1·587	Y
Placenta	Y	Y	Y	n/a	n/a	n/a	n/a	1·653	Y	n/a	n/a

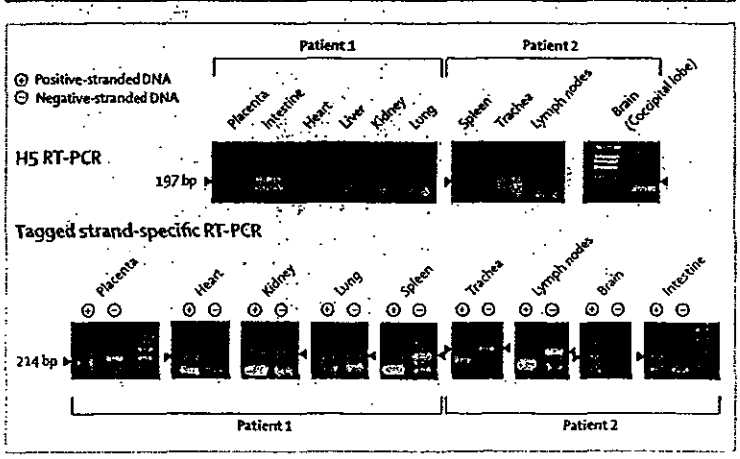

Figure 3: H5 RT-PCR and tagged strand-specific RT-PCR on selected organs
No positive band in RT-PCR seen for lymph nodes in patient 2.

Discussion

Our comprehensive investigation of the tissue tropism of H5N1 influenza virus, based on two adult autopsies and one fetal autopsy, focuses on the localisation of viral genomic sequences and antigens. We present evidence suggesting that the virus disseminates beyond the respiratory system. In addition to the lungs, viral sequences and antigens were found in the cerebral neurons and lymphocytes.

Presence of viral sequences and antigens in the CNS is consistent with the recent isolation of H5N1 virus from cerebrospinal fluid of a boy who died from encephalitis[4] with neurological symptoms commonly seen in patients with H5N1 influenza (Gao Zh, unpublished), including the two cases in this study. Brain neurons were found to be infected by the virus. We also saw regional variations in positive neuronal distribution and negative neurons next to positive neurons. Possible reasons might include differing densities of the avian influenza virus receptor in human beings, differences in blood supply pathways and nerve connections that allow virus-target cell contact, and differing viral loads and viral replication stages. The detection of positive-stranded RNA by RT-PCR and in-situ hybridisation could indicate active viral replication in the brain. The virus could reach the CNS by penetrating the blood-brain barrier or by invading the afferent fibres of the olfactory, vagal, trigeminal, and sympathetic nerves after replicating in the respiratory mucosa, as has been shown in animals.[24]

Our data imply that the virus also infects and actively replicates in the small intestines, which is consistent with previous studies.[5] The origin of infection in the intestines could be blood-borne, which is lent support by previous studies isolating live H5N1 virus from the serum[6] and plasma.[25] However, ingestion of infected respiratory secretion cannot be excluded as a possible route of infection, since H5N1 influenza viruses maintain sialidase activity despite the low pH in the upper digestive tract.[26] Although in-situ hybridisation, NASBA, and RT-PCR detected viral RNA in the intestines, immunohistochemistry for viral antigens was negative. This discrepancy is consistent with the findings of Uiprasertkul and colleagues,[5] although the reason is still unclear.

In-situ hybridisation and immunohistochemistry detected viral sequences and antigens in lymphocytes in the lymph nodes, and fetal macrophages in the placenta. Circulating mononuclear cells in the fetus and macrophages in the liver were found to harbour viral sequences. Previous in-vitro experiments have shown infection of macrophages by H5N1,[27,28] and ex-vivo experiments have shown that the virus attaches to alveolar macrophages in human lung tissue.[29] In addition to viraemia, infected immune cells could also carry the virus to extrapulmonary organs, which has been thought to participate in the pathogenesis of SARS.[29]

The virus localised to type II pneumocytes in the respiratory tract, which has also been reported previously.[5,29,30] However, with double labelling, we found viral sequences and antigens in both ciliated and non-ciliated epithelial cells of the trachea (figure 1C), contrasting with previous in-vivo and ex-vivo studies.[5,29] In cultures of human tracheobronchial epithelial cells, H5N1 influenza viruses have been reported to infect mainly ciliated cells, which express mainly avian influenza virus receptors (α-2,3-linked sialic acids), although a limited number of non-ciliated cells (<20% of all infected cells) have also been reported to be infected.[31,32] Some studies have detected only human influenza virus receptors (α-2,6-linked sialic acids) on non-ciliated cells,[32] whereas others also have found avian influenza virus receptors in these cells, albeit to a lesser extent.[31,33] Changes in receptor-binding properties of A/Anhui/1/2005 and A/Jiangxi/1/2005 viruses could, in theory, also account for the infection of non-ciliated cells. However, preliminary tests have not revealed any substantial changes in the receptor-binding sites of either virus, compared with previous H5N1 isolates.[12]

Notably, only a few scattered epithelial cells in the lungs were found to harbour the virus, contrasting with the severe and widespread histopathological changes in the lungs. Since this contrast was unexpected, lung tissue was sampled and analysed extensively, but the number of cells with viral localisation was consistently low in both patients. With the technique's very high detection sensitivity (close to 100%), the percentages of positive epithelial cells recorded in this study could be reasonable estimates of H5N1-infected cells. In view of the low number of infected cells in patient 1 and the absence of cells with positive signals after in-situ-hybridisation in patient 2, direct viral injury to the epithelial cells of the respiratory tract is, in our view, unlikely to cause such severe pathological changes. The lack of histopathological changes in the brain, despite our findings indicating active viral replication in the region, also suggests that viral replication might not be specifically pathogenic. Recent in-vitro and in-vivo studies have indicated that hyperinduction of cytokines and chemokines could take part in the pathogenesis of H5N1 influenza.[5,7,27,28,34]

Despite the high number of infected cells in the fetal respiratory system, we saw no evidence of severe damage to the fetal lungs, which greatly contrasts with the extensive damage found in the adult lungs. The absence of severe pulmonary damage (ie, high numbers of infected cells) in the fetus probably indicated an immunological naive status, which would be expected to result in low concentrations of the cytokines or chemokines to which the fetal lung tissues were exposed, and thus reduce or eliminate their induction of tissue damage. This theory is supported by in-vitro experiments[27] showing that H5N1-infected neonatal macrophages express much lower amounts of chemokines than H5N1-infected adult macrophages.

Although the intracellular distribution pattern of immunohistochemical signals conformed to our expectations, it did not for signals from in-situ hybridisation. Probes hybridised mainly in the nuclei of pneumocytes and in the cytoplasm of other organs. In mice infected with H5N1 influenza virus, nucleoprotein sense and antisense probes have also hybridised mainly in the cytoplasm of infected cells, although the reason for this finding is unclear.[34]

RNA analysis with RT-PCR and NASBA assays showed that H5-specific RNA was present in all tissues examined apart from the lymph nodes of patient 2, for which only NASBA showed positive result. This result could be due to the higher sensitivity of NASBA than that of RT-PCR. In fact, RT-PCR needed reamplification of the PCR products on the paraffin-embedded samples, which indicated a lower detecting sensitivity than NASBA.

RT-PCR and NASBA results were generally consistent with those of in-situ hybridisation and immunohistochemistry. However, viral RNA was also seen in viscera and some regions of the brain that showed negative results for both in-situ hybridisation and immunohistochemistry. A similar discrepancy has also been reported in a SARS study,[35] which was attributed either to very low copy numbers of RNA and protein in these organs that might not be detectable or to false-positive RT-PCR results. False-positive results might be caused by the presence of virus in blood perfusing the organs without actual viral replication in the tissue parenchyma.[35] Detection of positive-stranded

RNA in the lung, heart, intestines, placenta, brain, and trachea in our study could imply that viral replication occurs in these organs. The absence of corresponding negative-stranded RNA in the lung and heart could be due to a lower detecting sensitivity of RT-PCR for negative strands than for positive strands.

This study has shown the capacity for human vertical transmission of the H5N1 virus. Transplacental transmission of the H5N1 virus warrants careful investigation, since maternal infections with common human influenza virus are generally thought not to affect the fetus.[36] A sero-epidemiological study showed no evidence of transplacental transmission in pregnant women with human influenza infection.[37] Our placenta autopsy showed viral genomic sequences in cytotrophoblasts and resident macrophages; furthermore, the virus infected the fetus. Viraemia has been reported in avian influenza virus infections,[4,25] which is by contrast with the rare occurrence of viraemia in human influenza virus infections.[38] Therefore, the likelihood of virus reaching the uterus and placenta is probably higher in avian influenza than in human influenza.

The vertical transmission route of avian influenza virus could be similar to that of human cytomegalovirus, which also targets cytotrophoblasts and Hofbauer cells.[39] Two possible routes of transplacental transmission have been suggested:[39] transcytosis across syncytiotrophoblasts to cytotrophoblasts in chorionic villi, or via infection of invasive cytotrophoblasts in the uterine wall (which could be infected after contact with maternal blood). These infected cells subsequently transmit the virus to the anchoring chorionic villi and could then be transmitted to Hofbauer cells that enter the fetal circulation. The presence of viral sequences and antigens in cytotrophoblasts, Hofbauer cells, and circulating fetal mononuclear cells supports this theory.

We detected viral sequences in cytotrophoblasts of chorionic villi but not in syncytiotrophoblasts. Differences in virus receptor expression could explain why cytotrophoblasts are susceptible to avian influenza virus infections but not human influenza virus infections. Both syncytiotrophoblasts and cytotrophoblasts have been found to lack α-2,6-linked sialic acids,[40] but whether the placenta expresses α-2,3-linked sialic acids is unknown. The relative number of infected cells in the fetal lungs, as detected by in-situ hybridisation and immunohistochemistry, was substantially higher than in the two adults, which could be explained by the dominance of avian-influenza-virus receptors over human-influenza-virus receptors in the bronchial and alveolar epithelia during the pseudoglandular stage of lung histiogenesis (up to the 20th gestational week).[41]

Despite the long duration of the disease and antiviral treatment in patient 2, viral sequences and antigens were detected in the post-mortem tissues. This finding is different from a previous study.[7] The delayed clearance of viral antigens and sequences could be due to the immunosuppressive effect of the high-dose corticosteroids with which the patient had been treated for a long period before death. Since viral cultures were not done on post-mortem tissues, whether the detection of antigens and sequences indicates active viral replication is unclear. Positive results with in-situ hybridisation and RT-PCR have been reported in patients with SARS who died late in the course of disease.[20,35,42] However, these positive RT-PCR results have been ascribed to the presence of small amounts of residual genome, rather than to active viral replication.[35]

We have shown that the H5N1 virus spreads beyond the lungs, infecting both ciliated and non-ciliated epithelial cells of the trachea, the placenta, T lymphocytes in lymph nodes, and cerebral neurons. We also report evidence of transplacental transmission, resulting in infection of fetal organs. These newly obtained data are important in the clinical, pathological, and epidemiological investgation of human H5N1 infection, and have implications for public-health and health-care providers.

Contributors

JG initiated, designed, and coordinated the study, analysed the results, and took part in the writing of the manuscript. ZX took part in the autopsies, tissue collection, and routine pathology. ZhG was responsible for the clinical management and clinical data analysis. JLiu took part in the probe-design molecular biology, viral test, and the writing of the manuscript. JY did the immunohistochemistry and in-situ hybridisation. CK took part in the study design, result and literature analysis, and writing of the manuscript. ITL took part in the RT-PCR and NASBA, result analysis, and writing of the manuscript. JLu did the RT-PCR and NASBA. ZiG took part in the autopsies, tissue collection, routine pathology, and clinical data analysis. BZ did the molecular pathology, in-situ hybridisation, and probe design. MAM took part in the routine pathology, clinical data analysis, and writing of the manuscript. ML took part in the autopsies, tissue collection, and routine pathology. VMA did the fetoplacental pathology and molecular pathology. EG took part in routine pathology and tissue processing. ACHY designed and coordinated the RT-PCR and NASBA study, analysed the results, and took part in the writing of the manuscript. WIL had overall responsibility for the study design, and took part in the writing of the manuscript.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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別紙様式第2-1

No. 34

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	機構処理欄
				2007. 9. 16	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	Hamano M, Lim CK, Takagi H, Sawabe K, Kuwayama M, Kishi N, Kurane I, Takasaki T. Epidemiol Infect. 2007 Aug;135(6):974-7. Epub 2007 Jan 12.	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

○広島県の野生イノシシにおける日本脳炎ウイルスの検出
2004年11月から2005年2月にかけて、日本の西部に位置する広島県の野生イノシシから血清検体を25検体採取した。日本脳炎ウイルス(JEV)IgMキャプチャー及びIgG酵素免疫測定法(ELISA)、プラーク減少中和試験(PRNT)を行った。17検体(68%)がJEV中和抗体陽性だった。中和抗体陽性検体は全てIgG-ELISA陽性だった。1検体はIgMも陽性だった。この結果は、およそ70%の野生イノシシが抗JEV抗体陽性であったことを示し、野生イノシシがこの地域において日本脳炎ウイルス感染サイクルに関与している可能性を提起するものである。

報告企業の意見	今後の対応
広島県の野生イノシシのうち、およそ70%が日本脳炎ウイルス抗体陽性であり、野生イノシシがこの地域において日本脳炎ウイルス感染サイクルに関与している可能性が提起されたとの報告である。	今後も情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

(5-1)

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SHORT REPORT
Detection of antibodies to Japanese encephalitis virus in the wild boars in Hiroshima prefecture, Japan

M. HAMANO[1], C. K. LIM[1], H. TAKAGI[2], K. SAWABE[3], M. KUWAYAMA[4], N. KISHI[5], I. KURANE[1]* AND T. TAKASAKI[1]

[1] *Department of Virology 1, National Institute of Infectious Diseases, Tokyo, Japan*
[2] *Division of Biosafety Control and Research, National Institute of Infectious Diseases, Tokyo, Japan*
[3] *Department of Medical Entomology, National Institute of Infectious Diseases, Tokyo, Japan*
[4] *Division of Microbiology II, Hiroshima Prefectural Institute of Health and Environment, Hiroshima, Japan*
[5] *Hiroshima Prefectural Livestock Technological Research Center, Hiroshima, Japan*

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SUMMARY

Serum specimens were collected from 25 wild boars in Hiroshima prefecture located in the western region of Japan from November 2004 to February 2005. The sera were tested for antibodies to Japanese encephalitis virus (JEV) by IgM capture and IgG enzyme-linked immunosorbent assays (ELISA), and plaque reduction neutralization test. Seventeen samples (68%) were positive for neutralizing antibody to JEV. All the neutralizing antibody-positive samples were positive for IgG-ELISA. One was also positive for IgM. The results indicate that approximately 70% of the wild boars were positive for anti-JEV antibody, and raises the possibility that wild boars may play a role in the infectious cycle of JEV in this region.

Japanese encephalitis virus (JEV) is an arbovirus, and a member of the genus *Flavivirus*, family Flaviviridae. JEV was first isolated in 1935 in Tokyo, Japan, from the human brain of a fatal Japanese encephalitis (JE) case. JEV is transmitted by *Culex* mosquitoes in an epizoonotic cycle [1–3], and is a serious cause of human morbidity and mortality in Asia.

JEV is also a veterinary problem, especially for horses. Many species of animals, such as pigs, horses, dogs, chickens, ducks, and reptiles, are infected in the wild. Pigs are the major amplifying hosts of JEV, although infection usually does not induce clinical symptoms. Pigs and birds develop high-titre viraemia which provides an excellent source of infection for mosquitoes. In parts of Asia, pigs are an important source of viral amplification and significantly enhance human exposure and infection [3].

In recent years, the numbers of JEV-infected pigs and the households that breed pigs have decreased [4]. However, in 2000, the JE genome was detected in cerebrospinal fluid specimens from four patients with aseptic meningitis in Hiroshima prefecture, Japan [5]. Moreover, three JE cases occurred in 2002 for the first time in 12 years [6]. Pig farms are usually located far away from residential areas. It is, thus, possible that animals other than domestic pigs are playing a role as an amplifier and reservoir for JEV. We suspected that wild boars might play a role as an amplifier for transmission of JEV to humans. From 2004 to 2005, serum samples were collected from the wild boars in Hiroshima prefecture and analysed for anti-JEV IgM and IgG antibodies.

JEV (JEV/sw/Hiroshima/25/2002 strain, NCBI accession no. AB231621) which was isolated from a

* Author for correspondence: I. Kurane M.D., Department of Virology 1, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.
(Email: kurane@nih.go.jp)

Table 1. *Serum samples used in the study and results of antibody assays*

Sample no.	Date	Sex	Body weight (kg)	PRNT50 titre	IgG-ELISA (Index)	IgM-ELISA (Index)
22	22 Dec. 2004	M	80	2560	+(1·34)	−(0·80)
24	19 Jan. 2005	F	24	2560	+(1·88)	−(0·70)
35	6 Feb. 2005	F	65	160	+(1·21)	+(1·33)
33	4 Feb. 2005	M	25	160	+(1·07)	−(0·87)
16	2 Jan. 2005	M	95	160	+(2·66)	−(0·51)
21	21 Dec. 2004	M	30	40	+(1·47)	−(0·72)
10	17 Dec. 2004	M	40	40	+(3·35)	−(0·53)
17	8 Jan. 2005	M	90	40	+(2·78)	−(0·68)
1	20 Nov. 2004	F	60	10	+(2·21)	−(0·55)
8	8 Dec. 2004	M	32	10	+(1·70)	−(0·47)
2	21 Nov. 2004	M	110	10	+(2·43)	−(0·62)
18	9 Jan. 2005	M	95	10	+(1·68)	−(0·46)
29	24 Jan. 2005	M	70	10	+(1·39)	−(0·69)
3	30 Nov. 2004	M	70	10	+(1·93)	−(0·47)
19	10 Jan. 2005	F	55	10	+(1·90)	−(0·43)
34	5 Feb. 2005	M	70	10	+(1·08)	−(0·76)
28	22 Jan. 2005	M	65	10	+(1·52)	−(0·61)
6	19 Nov. 2004	M	40	<10	−(0·35)	−(0·56)
7	8 Dec. 2004	F	30	<10	−(0·57)	−(0·74)
9	9 Dec. 2004	F	40	<10	−(0·38)	−(0·37)
11	17 Dec. 2004	M	100	<10	+(2·65)	−(0·74)
14	26 Dec. 2004	M	90	<10	−(0·40)	−(0·45)
31	19 Jan. 2005	F	26	<10	−(0·57)	−(0·74)
30	25 Jan. 2005	F	70	<10	+(1·07)	−(0·79)
39	8 Feb. 2005	M	27	<10	−(0·58)	−(0·79)

Total number is 25 (n=25). + and − indicate positive and negative, respectively. The tested samples were determined to be IgG- or IgM-positive when index values were higher than 1·0.

pig in Hiroshima prefecture in 2002 was used in the present study. Vero cells (9013 cell; purchased from Japanese Science Research Resources Bank) were maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% foetal bovine serum (FBS, ICN Biomedicals Inc., OH, USA), penicillin & streptomycin (P&S, Gibco, NY, USA) and non-essential amino acids (NEAA, Gibco, NY, USA) in 5% CO_2 at 37 °C.

Wild boars (*Sus leucomystax*) were hunted by the members of the Hiroshima hunting club in Hiroshima prefecture from 20 November 2004 to 8 February 2005 (Table 1). The areas were located in the north-east of Hiroshima prefecture. Blood specimens were collected from the heart of hunted wild boars after death, and sent to the Laboratory of Vector-borne Viruses, Department of Virology 1, National Institute of Infectious Diseases, Tokyo, as soon as possible under refrigeration. These wild boars appeared in good health. The sera were separated and kept at −20 °C until use.

IgM-capture ELISA was performed as previously reported [7, 8]. Briefly, sera were diluted at 1:100 with phosphate buffered saline [PBS(−), Sigma-Aldrich, MO, USA] including 0·1% BSA and 0·05% Tween-20. Diluted serum samples were added to 96-well plates coated with anti-porcine IgM antibody (μ-chain specific, Serotec, UK) and incubated at 37 °C for 60 min. The plates were washed with PBS(−) including 0·05% Tween-20 (TPBS) six times. After washing, JEV antigen (JaGAr01 strain) was added to each well and reacted at room temperature for 2 h. The plate was washed with TPBS six times and reacted with 1:500 diluted horseradish peroxidase-conjugated, flavivirus-cross-reactive mAb, D1-4G2-4-15 (4G2) [8] at 37 °C for 60 min. The plates were washed with TPBS six times. Tetramethylbenzidine (TMB; Moss Inc., CA, USA) was added and incubated in the dark at room temperature for 10 min. Stop solution (1 N H_2SO_4) was added and optical density (OD) (450 nm) was measured by an ELISA reader, ELX-800 (Bio-Tec Instruments Inc., VT, USA).

IgG-ELISA was performed as follows. Ninety-six-well ELISA plates (Nunc, C8 Polysorp Nunc immuno module, Roskilde, Denmark) were coated with inactivated and purified JEV (Beijing-1 strain) antigen in PBS(–). Serum samples diluted at 1:100 were added and reacted at room temperature for 90 min. Plates were washed with TPBS six times and reacted with 1:10000 diluted, horseradish peroxidase-conjugated anti-pig IgG antibody (Bethyl, TX, USA) in PBS(–) at room temperature for 60 min. The plates were washed with TPBS six times. TMB was added and plates were incubated in the dark at room temperature for 10 min. Stop solution was added and the OD (450 nm) was read by an ELISA reader ELX-800. The index value was calculated by the formula: average OD value of tested sample divided by average OD value of the positive control pig serum. This positive control pig serum demonstrated OD values marginally higher than the average OD +3 S.D. obtained using JE antibody-negative porcine sera in IgM-ELISA and IgG-ELISA. When the index value was higher than 1·0, the tested sample was determined to be antibody positive.

Plaque reduction neutralization test (PRNT) was performed as follows. Briefly, Vero cell monolayer was prepared in EMEM supplemented with 10% FBS, P&S and NEAA on the six-well plates (Corning, NY, USA). The serum samples were four-fold serially diluted from 1:10 to 1:2560 and mixed with same volume of diluted JEV at 37 °C for 90 min. After incubation, the mixtures were inoculated onto Vero cell monolayer. After the plates were incubated at 35 °C for 60 min, EMEM containing 2% FBS and 1% methyl cellulose (Wako, Japan) was overlaid in the wells. The plates were incubated at 35 °C in 5% CO_2 for 4 days. Cells were fixed with 3·7% formaldehyde (Wako, Japan), stained with Methylene Blue tetrahydrate solution and plaque numbers were counted as previously described [9]. The reduction percent at each serum dilution was calculated. Neutralizing antibody titre indicates the highest dilution which demonstrated more than 50% of the reduction.

Of 25 serum samples from wild boars, 17 samples were positive for JEV-specific neutralizing antibody; the titres ranging from 20 to >2560 (Table 1). These 17 samples were also determined to be positive by IgG-ELISA. Two samples were neutralizing Ab-negative and IgG-ELISA-positive. We considered the serum samples positive for both neutralizing Ab and IgG-ELISA Ab as positive. According to this criterion, 68% of the wild boars were positive for JEV antibody. Only one sample (no. 35) was positive for IgM, suggesting a recent primary infection.

The numbers of Japanese encephalitis human cases have been less than 10 since 1992 in Japan. Haemagglutination inhibition (HI) antibody to JEV is positive in over 80% of sentinel pigs in the western regions of Japan every year. In 2002, one JE patient was reported in Hiroshima city [5, 10]. Concerning the environment of the patient's residence, there are paddy fields but no pig farms nearby within a 10 km radius (personal communication from the physician). Recently, wild boars frequently prowled around in the residential areas in Hiroshima prefecture. Twenty-five serum samples collected from wild boars in Hiroshima prefecture were examined on IgM, IgG and neutralizing antibody. Only one sample (no. 35) was positive for IgM, IgG and neutralizing antibody. Virus-specific IgM antibody appears in the serum during the acute phase after viral infection but is present only for an average of 3 weeks [11]. Sixteen samples were positive for IgG-ELISA and neutralizing antibodies, but negative for IgM. Two wild boars (nos. 22 and 24) demonstrated a high level (titre of 2560) of neutralizing antibody, suggesting multiple infections. It was reported that high levels of viraemia were detected in pigs on the day after inoculation and lasted about 4 days [12–14]. In our study, PCR analysis was performed using sample sera that were taken in the winter season. JEV is not active in the winter season. Accordingly, viral RNA was not detected and it was difficult to detect JEV in the present study (data not shown). The results suggest that wild boars that scavenge for food around human residences could be infected with JEV based on the results of IgG-ELISA, IgM-ELISA and PRNT assays.

It is known that pigs (*Sus scrofa* var. *domestica*; Yorkshire and Berkshire) are a reservoir for JEV [12–16]. Wild boar is a closely related species to the domestic pig; therefore, it is reasonable to hypothesize that wild boars are also a reservoir for JEV in addition to pigs. There was a report of seroepidemiology of JEV infection in wild boars (*Sus barbatus*) in Singapore. The authors suggested that JEV might still be transmitted actively in Singapore, although pig farming had been phased out [17]. In Japan, although the number of pigs has been maintained at around 10000000 heads since 1995, the number of pig farms has been greatly reduced to around 20000 farms. Moreover, the number of wild-caught boars in Japan has increased from 16354 heads in 1995 to 47629 in

2000, according to the data from the Ministry of the Environment, Japan. It is likely that more wild boars live close to humans in some areas in Japan. This raises the possibility that wild boars could act as an amplifying host, like the domestic pig, and in turn provide a reservoir for mosquitoes (*Culex tritaeniorhynchus*). Further studies are required to establish the viral titres in wild boar to assess their ability to act as an amplifying host. The present study demonstrated that the majority of wild boars are positive for JEV antibodies in Hiroshima prefecture in the western region of Japan where human JEV cases were reported in 2002.

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DECLARATION OF INTEREST

None.

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B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料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(2007/9/1～2007/11/30)

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
70101	2007/09/19	日本メジフィジックス	放射性医薬品基準テクネチウム大凝集人血清アルブミン(99mTc)	テクネチウム大凝集人血清アルブミン(99mTc)	生物学的製剤基準人血清アルブミン	日本	有効成分	有	無	無
70102	2007/09/21	化学及血清療法研究所	フィブリノゲン加第XIII因子	アプロチニン	ウシ肺臓	ウルグアイ	有効成分	無	無	無
70103	2007/09/21	日本製薬	乾燥ポリエチレングリコール処理人免疫グロブリン	ポリエチレングリコール処理人免疫グロブリンG	人血液	日本	有効成分	有	無	無
70104	2007/09/21	日本製薬	乾燥濃縮人アンチトロンビンIII	人アンチトロンビンIII	人血液	日本	有効成分	有	無	無
70105	2007/09/21	日本製薬	トロンビン	トロンビン	人血液	日本	有効成分	有	無	無
70106	2007/09/21	日本製薬	人血清アルブミン 加熱人血漿たん白	人血清アルブミン	人血液	日本	有効成分	有	無	無
70107	2007/09/21	ベネシス	①②ポリエチレングリコール処理人免疫グロブリン ③人免疫グロブリン	人免疫グロブリンG	人血液	①日本 ②③米国	有効成分	有	有	無
70108	2007/09/21	ベネシス	①②③人血清アルブミン ④乾燥濃縮人血液凝固第VIII因子 ⑤乾燥濃縮人血液凝固第IX因子	人血清アルブミン	人血液	①②④⑤日本、 ③米国	①②③有効成分、 ④⑤添加物	有	無	無
70109	2007/09/21	ベネシス	乾燥濃縮人血液凝固第VIII因子	血液凝固第VIII因子	人血液	日本	有効成分	有	無	無
70110	2007/09/27	CSLベーリング	①～③人血清アルブミン ④人血液凝固第XIII因子 ⑤⑥フィブリノゲン加第XIII因子	人血清アルブミン	ヒト血液	①④⑤⑥米国、ドイツ、オーストリア ②③米国	①～③有効成分、 ④～⑥添加物	有	有	有
70111	2007/09/27	CSLベーリング	フィブリノゲン加第XIII因子	アプロチニン液	ウシ肺	ウルグアイ、ニュージーランド	有効成分	無	無	無
70112	2007/09/28	日本赤十字社	(製造承認書に記載なし)	合成血	人血液	日本	有効成分	有	無	有
70113	2007/09/28	日本赤十字社	人血清アルブミン	人血清アルブミン	人血液	日本	有効成分	有	無	有
70114	2007/09/28	バクスター	乾燥人血液凝固因子抗体迂回活性複合体	乾燥人血液凝固因子抗体迂回活性複合体	人血漿	米国	有効成分	有	無	無
70115	2007/09/28	バクスター	乾燥濃縮人血液凝固第VIII因子	乾燥人血液凝固第VIII因子	人血漿	米国	有効成分	有	無	無
70116	2007/09/28	バクスター	乾燥濃縮人血液凝固第VIII因子	人血清アルブミン	人血漿	米国	添加物	有	無	無
70117	2007/10/05	化学及血清療法研究所	人免疫グロブリン ヒスタミン加人免疫グロブリン製剤	免疫グロブリン	ヒト血液	日本	有効成分	有	無	無
70118	2007/10/05	化学及血清療法研究所	フィブリノゲン加第XIII因子	人フィブリノゲン	ヒト血液	日本	有効成分	有	無	無
70119	2007/10/05	化学及血清療法研究所	フィブリノゲン加第XIII因子	人血液凝固第XIII因子	ヒト血液	日本	有効成分	有	無	無
70120	2007/10/05	化学及血清療法研究所	①フィブリノゲン加第XIII因子 ②乾燥濃縮人活性化プロテインC ③トロンビン	トロンビン	ヒト血液	日本	①③有効成分 ②製造工程	有	無	無

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
70121	2007/10/05	化学及血清療法研究所	①ボルヒール ②乾燥濃縮人活性化プロテインC ③乾燥濃縮人血液凝固第Ⅸ因子 ④⑧乾燥スルホ化人免疫グロブリン ⑤⑥⑦⑩⑪人血清アルブミン ⑨乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	ヒト血液	日本	①～④、⑧ ⑨添加物 ⑤～⑦、⑩ ⑪有効成分	有	無	無
70122	2007/10/05	化学及血清療法研究所	乾燥濃縮人活性化プロテインC	プロテインC	ヒト血液	日本	有効成分	有	無	無
70123	2007/10/05	化学及血清療法研究所	乾燥濃縮人活性化プロテインC 乾燥濃縮人血液凝固第Ⅸ因子	マウス由来モノクローナル抗体	マウス脾臓	日本	製造工程	無	無	無
70124	2007/10/23	化学及血清療法研究所	抗HBs人免疫グロブリン	抗HBs人免疫グロブリン	ヒト血液	米国	有効成分	有	無	無
70125	2007/10/24	バイエル薬品	①加熱人血漿たん白 ②③オクトコグ　アルファ(遺伝子組換え)	加熱人血漿たん白	ヒト血液	米国	①有効成分 ②③製造工程	有	有	無
70126	2007/10/24	バイエル薬品	pH4処理酸性人免疫グロブリン	人免疫グロブリンG	ヒト血液	米国	有効成分	有	有	無
70127	2007/10/24	バイエル薬品	①人血清アルブミン ②オクトコグ　アルファ(遺伝子組換え) ③インターフェロンベータ-1b(遺伝子組換え)	人血清アルブミン	ヒト血液	米国	①有効成分 ②製造工程 ③添加物	有	有	無
70128	2007/10/24	バイエル薬品	オクトコグ　アルファ(遺伝子組換え)	ウシインスリン	ウシ膵臓	米国	製造工程	有	無	無
70129	2007/10/24	バイエル薬品	オクトコグ　アルファ(遺伝子組換え)	ヒトトランスフェリン	ヒト血液	米国	製造工程	有	無	無
70130	2007/10/24	CSLベーリング	フィブリノゲン加第XIII因子 人血液凝固第XIII因子	人血液凝固第XIII因子	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	有
70131	2007/10/24	CSLベーリング	フィブリノゲン加第XIII因子	フィブリノゲン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
70132	2007/10/24	CSLベーリング	抗破傷風人免疫グロブリン	破傷風抗毒素	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
70133	2007/10/24	CSLベーリング	フィブリノゲン加第XIII因子	トロンビン末	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
70134	2007/10/24	CSLベーリング	フィブリノゲン加第XIII因子	アンチトロンビン	ヒト血液	米国、ドイツ、オーストリア	製造工程	有	無	有
70135	2007/10/24	CSLベーリング	人免疫グロブリン	人免疫グロブリン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無
70136	2007/10/25	日本メジフィジックス	放射性医薬品基準人血清アルブミン五酢酸テクネチウム(99mTc)注射液	人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc)注射液	生物学的製剤基準人血清アルブミン	日本	有効成分	無	無	無
70137	2007/10/26	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	ウサギIgG	ウサギ血液	日本	製造工程	無	無	無
70138	2007/10/26	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	ヤギIgG	ヤギ血液	オーストラリア	製造工程	無	無	無
70139	2007/10/26	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	マウスモノクローナル抗体	マウス脾臓細胞と骨髄腫細胞のハイブリ[illegible]	イギリス	製造工程	無	無	無

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
70140	2007/10/26	ベネシス	乾燥抗HBs人免疫グロブリン ポリエチレングリコール処理抗HBs人免疫グロブリン	抗HBs抗体	人血液	米国	有効成分	有	無	無
70141	2007/10/26	日本製薬	乾燥抗HBs人免疫グロブリン	抗HBs抗体	人血液	米国	有効成分	有	無	無
70142	2007/10/26	日本製薬	乾燥抗破傷風人免疫グロブリン	破傷風抗毒素	人血液	米国	有効成分	有	無	無
70143	2007/10/26	日本赤十字社	新鮮凍結人血漿	新鮮凍結人血漿	人血液	日本	有効成分	有	有	有
70144	2007/10/26	日本赤十字社	人血小板濃厚液	人血小板濃厚液	人血液	日本	有効成分	有	有	有
70145	2007/10/26	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液	人血液	日本	有効成分	有	有	有
70146	2007/10/26	日本赤十字社	乾燥濃縮人血液凝固第Ⅷ因子	乾燥濃縮人血液凝固第Ⅷ因子	人血液	日本	有効成分	有	無	無
70147	2007/10/26	日本赤十字社	乾燥濃縮人血液凝固第Ⅷ因子	人血清アルブミン	人血液	日本	添加物	有	無	無
70148	2007/10/26	日本赤十字社	人免疫グロブリン	人免疫グロブリン	人血液	日本	有効成分	有	無	無
70149	2007/10/26	日本赤十字社	pH4処理酸性人免疫グロブリン	pH4処理酸性人免疫グロブリン	人血液	日本	有効成分	有	無	無
70150	2007/10/31	バクスター	人血清アルブミン	人血清アルブミン	人血漿	米国	有効成分	無	有	無
70151	2007/11/16	ベネシス	トロンビン	トロンビン	人血液	日本	有効成分	有	無	無
70152	2007/11/16	ベネシス	乾燥濃縮人血液凝固第Ⅸ因子	血液凝固第Ⅸ因子	人血液	日本	有効成分	有	無	無
70153	2007/11/16	ベネシス	乾燥人フィブリノゲン	凝固性たん白質	人血液	日本	有効成分	有	無	無
70154	2007/11/16	ベネシス	乾燥抗D(Rho)人免疫グロブリン	抗D(Rho)抗体含有人免疫グロブリンG	人血液	米国	有効成分	有	無	無
70155	2007/11/16	ベネシス	乾燥濃縮人アンチトロンビンⅢ	人アンチトロンビンⅢ	人血液	日本	有効成分	有	無	無
70156	2007/11/21	化学及血清療法研究所	乾燥ペプシン処理人免疫グロブリン	ペプシン処理人免疫グロブリンG分屑	ヒト血液	日本	有効成分	有	無	無
70157	2007/11/21	化学及血清療法研究所	乾燥ペプシン処理人免疫グロブリン	ペプシン	ブタ胃粘膜	米国、カナダ.	製造工程	無	無	無
70158	2007/11/21	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG	ヒト血液	ドイツ	有効成分	有	有	有
70159	2007/11/21	CSLベーリング	ペプシン処理人免疫グロブリンG	ペプシン処理人免疫グロブリンG	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	無
70160	2007/11/21	CSLベーリング	ペプシン処理人免疫グロブリンG	ペプシン	ブタの胃	米国	製造工程	無	無	無
70161	2007/11/21	CSLベーリング	人免疫グロブリン	人免疫グロブリン	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	無

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使用措置
70162	2007/11/21	富士フイルムRIファーマ	テクネチウム人血清アルブミン(99mTc)	テクネチウム人血清アルブミン(99mTc)	ヒト血液	日本	有効成分	有	無	無
70163	2007/11/26	日本製薬	人免疫グロブリン	免疫グロブリンG	人血液	日本	有効成分	有	無	無
70164	2007/11/26	日本製薬	乾燥抗D(Rho)人免疫グロブリン	抗D(Rho)抗体	人血液	米国	有効成分	有	無	無
70165	2007/11/29	日本赤十字社	解凍人赤血球濃厚液	解凍人赤血球濃厚液	人血液	日本	有効成分	有	無	有

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６回	6-1	感染症および寄生虫症	伝染性紅斑	日本	男	1	2005 年 10 月 11 日	回復	症例報告	当該製品	未完了報告：2005 年 12 月 1 日 識別番号：A-05000194
	5-1	肝胆道系障害	肝機能異常	日本	男	11 ヵ月	2005 年 4 月 5 日	回復	症例報告	当該製品	完了報告：2005 年 9 月 26 日 識別番号：A-05000095 第 5 回報告の「ウイルス性肝炎」から副作用・感染症名が「肝機能障害」に変更になった。
第５回	5-1	感染症および寄生虫症	ウイルス性肝炎	日本	男	11 ヵ月	2005 年 4 月 5 日	回復	症例報告	当該製品	未完了報告：2005 年 7 月 27 日 識別番号：A-05000095
第３回	3-1	感染症および寄生虫症	ロタウイルス胃腸炎	日本	女	1	2004 年 4 月 20 日	軽快	症例報告	当該製品	

MedDRA/J Version10.0 使用

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別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第９回	9-1	感染症および寄生虫症	伝染性紅斑	日本	男	10	2007 年 1 月 24 日	回復	症例報告	当該製品	
	8-1	全身障害および投与局所様態	発熱	日本	男	11 ヵ月	2006 年 7 月 8 日	回復	症例報告	当該製品	完了報告：2007 年 2 月 2 日 識別番号：A-06000119 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
		皮膚および皮下組織障害	発疹				2006 年 7 月 11 日	回復			
		肝胆道系障害	肝障害				2006 年 7 月 10 日	回復			
	8-3	皮膚および皮下組織障害	全身紅斑	日本	男	1	2007 年 1 月 2 日	回復	症例報告	当該製品	取下げ報告：2007 年 2 月 15 日 識別番号：A-06000214
第８回	8-1	全身障害および投与局所様態	発熱	日本	男	11 ヵ月	2006 年 7 月 8 日	回復	症例報告	当該製品	未完了報告①：2006 年 9 月 19 日 未完了報告②：2006 年 10 月 3 日 未完了報告③：2006 年 10 月 18 日 識別番号：A-06000119 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
		皮膚および皮下組織障害	発疹				2006 年 7 月 11 日	回復			
		肝胆道系障害	肝障害				2006 年 7 月 10 日	回復			
	8-2	感染症および寄生虫症	伝染性紅斑	日本	女	6	2006 年 11 月 13 日	回復	症例報告	当該製品	
	8-3	皮膚および皮下組織障害	全身紅斑	日本	男	1	2007 年 1 月 2 日	回復	症例報告	当該製品	未完了報告：2007 年 1 月 31 日 識別番号：A-06000214 ウイルス感染の可能性も完全に否定できないとのことにより感染症として取り扱う。
第７回	6-1	感染症および寄生虫症	伝染性紅斑	日本	男	1	2005 年 10 月 11 日	回復	症例報告	当該製品	取下げ報告：2006 年 3 月 24 日 識別番号：A-05000194

70107	2007/09/21	70537	ベネシス	①②ポリエチレングリコール処理人免疫グロブリン ③[illegible]免疫グロブリン	人免疫グロブリンG	ヴェノ

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別紙様式第4

感染症発生症例一覧

別紙(5)

	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9回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 報告書提出日2007年7月24日
	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	1	臨床検査	C型肝炎陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000032 報告書提出日2007年2月23日
	2	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000032 完了報告書提出日2007年3月30日
	2	臨床検査	C型肝炎陽性	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000032 完了報告書提出日2007年3月30日
第8回	1	感染症および寄生虫症	B型肝炎	ドイツ	女	27	2006年6月	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	1	臨床検査	B型肝炎表面抗原陽性	ドイツ	女	27	2006年6月	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	1	臨床検査	抗HBs抗体陽性	ドイツ	女	27	2006年6月	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	1	臨床検査	抗HBcIgG抗体陽性	ドイツ	女	27	2006年6月	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	1	臨床検査	抗HBe抗体陽性	ドイツ	女	27	2006年6月	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	2	感染症および寄生虫症	C型肝炎	ドイツ	男	37	不明	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例
	3	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　提出日2006年12月7日
	3	臨床検査	C型肝炎抗体陽性	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　提出日2006年12月7日
	3	臨床検査	C型肝炎RNA陽性	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　提出日2006年12月7日
	3	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　追加報告提出日2006年12月20日
	3	臨床検査	C型肝炎抗体陽性	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　追加報告提出日2006年12月20日
	3	臨床検査	C型肝炎RNA陽性	ドイツ	女	41	2006/11/21	不明	その他	外国製品	弊社血漿分画製剤と原料血漿が同様の可能性のある症例 識別番号3-06000029　追加報告提出日2006年12月20日

70110	2007/09/27	70550	CSLベーリング	①～③人血清アルブミン ④人血液凝固第XIII因子 ⑤⑥フィブリノゲン加第XIII因子	人血清アルブミン

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6回	6-1	感染症および寄生虫症	肝炎ウイルスキャリアー	フランス	男	不明	不明	不明	症例報告	外国製品	2005/11/15、3-05000462
第4回	4-1	感染症および寄生虫症	細菌性関節炎	フランス	男	不明	2004/3	不明	症例報告	外国製品	2004/09/28、3-04000067
	4-2	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
	4-3	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
	4-4	感染症および寄生虫症	パルボウイルス感染	日本	不明	不明	不明	不明	その他	当該製品	2005/01/27、5-04000014
第3回	3-1	臨床検査	C型肝炎陽性	カナダ	男	43歳	2003/8/20	不明	症例報告	外国製品	2004/04/20、3-04000004
	2-1	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/08/05、3-03000002
第2回	2-1	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/01/13、3-03000002

MedDRAバージョン10.0により集計した。

第9回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第8回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第7回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第5回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第3回症例番号2-1は，第2回症例番号2-1において報告したものの追加報告。

第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

70125	2007/10/24	70644	バイエル薬品	①加熱人血漿たん白 ②③オクトコグ　アルファ(遺伝子組換え)	加熱人血漿たん白

別紙様式第4

別紙5

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第9回	9-1	感染症および寄生虫症	ＪＣウイルス感染	カナダ	男	18歳	2007/2	死亡	症例報告	外国製品	2007/07/11、3-07000006
	9-2	臨床検査	Ｃ型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/07/25、3-07000007
	8-1	感染症および寄生虫症	ブドウ球菌感染	カナダ	女	50歳	2006/10/20	不明	症例報告	外国製品	2007/03/02、3-06000024
	8-2	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	46歳	2006/7/17	不明	症例報告	外国製品	2007/03/02、3-06000030
	9-5	臨床検査	Ｃ型肝炎ウイルス	アメリカ	男	47歳	不明	未回復	症例報告	外国製品	2007/07/17、3-07000008
第8回	8-1	感染症および寄生虫症	ブドウ球菌感染	カナダ	女	50歳	2006/10/20	不明	症例報告	外国製品	2006/11/14、3-06000024
	8-2	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	46歳	2006/7/17	不明	症例報告	外国製品	2007/02/20、3-06000030
第7回	7-1	臨床検査	抗ＨＢｓ抗体陽性	アメリカ	男	17歳	2006/2/18	不明	症例報告	外国製品	2006/04/13、3-05000685
	7-1	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	17歳	2006/2	不明	症例報告	外国製品	2006/04/13、3-05000685
	7-2	感染症および寄生虫症	Ｂ型肝炎	カナダ	女	13歳	2005/12/1	不明	症例報告	外国製品	2006/06/28、3-06000002
	7-2	臨床検査	Ｂ型肝炎陽性	カナダ	女	13歳	2005/7/22	不明	症例報告	外国製品	2006/06/28、3-06000002
第6回	6-1	感染症および寄生虫症	ウエストナイルウイルス感染	アメリカ	男	43歳	不明	不明	症例報告	外国製品	2006/02/15、3-05000574
第5回	4-8	臨床検査	Ｂ型肝炎陽性	アメリカ	男	66歳	2005/1/13	不明	症例報告	外国製品	2005/03/30、3-04000093
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/11/1	不明	症例報告	外国製品	2005/06/28、3-04000022
	2-4	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/8/30	不明	症例報告	外国製品	2005/03/09、3-03000017
	4-9	臨床検査	Ｂ型肝炎抗体陽性	アメリカ	女	32歳	不明	不明	症例報告	外国製品	2005/03/28、3-04000097
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	26歳	不明	不明	症例報告	外国製品	2005/06/28、3-04000022
第4回	3-1	臨床検査	Ｂ型肝炎抗体陽性	カナダ	女	56歳	2004/3/10	不明	症例報告	外国製品	2004/09/17、3-04000007
	3-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/10/10	不明	症例報告	外国製品	2004/09/03、3-04000025
	3-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/8/25	不明	症例報告	外国製品	2004/08/31、3-04000026
	3-6	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/6/20	不明	症例報告	外国製品	2004/09/03、3-04000027
	3-8	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/10/01、3-04000028
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000022
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000022
	4-7	感染症および寄生虫症	単純ヘルペス	アメリカ	女	不明	不明	不明	症例報告	外国製品	2005/01/12、3-04000089

別紙5

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第4回	4-8	臨床検査	Ｂ型肝炎陽性	アメリカ	不明	不明	不明	不明	症例報告	外国製品	2005/02/02、3-04000093
	4-9	臨床検査	Ｂ型肝炎抗体陽性	アメリカ	女	不明	不明	不明	症例報告	外国製品	2005/02/23、3-04000097
第3回	3-1	肝胆道系障害	肝炎	カナダ	女	56歳	2004/3/12	不明	症例報告	外国製品	2004/05/07、3-04000007
	3-2	臨床検査	Ｂ型肝炎陽性	アメリカ	女	48歳	2004/3	未回復	症例報告	外国製品	2004/06/21、3-04000017
	3-3	臨床検査	Ｃ型肝炎陽性	アメリカ	女	48歳	2004/3	未回復	症例報告	外国製品	2004/06/21、3-04000017
	3-4	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/10/10	不明	症例報告	外国製品	2004/07/20、3-04000025
	2-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	25歳	2003/9/26	回復	症例報告	外国製品	2004/07/06、3-03000010
	3-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/8/25	不明	症例報告	外国製品	2004/08/31、3-04000026
	3-6	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/6/20	不明	症例報告	外国製品	2004/07/20、3-04000027
	2-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/5/26	回復	症例報告	外国製品	2004/08/09、3-03000008
	3-8	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000028
	3-9	感染症および寄生虫症	Ｃ型肝炎	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/05/31、3-04000011
	3-10	感染症および寄生虫症	Ｂ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/06/30、3-04000022
	3-10	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	不明	不明	不明	症例報告	外国製品	2004/06/30、3-04000022
第2回	2-1	臨床検査	ＨＴＬＶ－１検査陽性	日本	男	26歳	2003/12	不明	症例報告	当該製品	2004/02/26、1-03000112
	2-2	感染症および寄生虫症	マイコプラズマ感染	日本	女	不明	2003/7	不明	症例報告	当該製品	2004/01/14、1-03000016
	2-3	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	不明	2003/5	不明	症例報告	外国製品	2004/02/23、3-03000008
	2-4	感染症および寄生虫症	Ｃ型肝炎	アメリカ	男	26歳	2002/8/30	不明	症例報告	外国製品	2004/01/13、3-03000017
	2-5	臨床検査	ＨＴＬＶ－１検査陽性	カナダ	女	25歳	不明	不明	症例報告	外国製品	2004/01/05、3-03000010

MedDRAバージョン10.0により集計した。

第9回症例番号8-1, 8-2は、それぞれ第8回症例番号8-1, 8-2において報告したものの追加報告。

第5回症例番号4-8, 3-10, 2-4, 4-9, 3-10は、それぞれ第4回、第3回および第2回症例番号4-8, 3-10, 2-4, 4-9, 3-10において報告したものの追加報告。

第4回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10は，それぞれ第3回症例番号3-1, 3-3, 3-5, 3-6, 3-8, 3-10において報告したものの追加報告。

第4回症例番号3-1の有害事象名は，第3回報告時では「肝炎」であったが追加情報により，「Ｂ型肝炎抗体陽性」に変更された。

第3回症例番号2-5, 2-3は，それぞれ第2回症例番号2-5, 2-3において報告したものの追加報告。

第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

70126	2007/10/24	70645	バイエル薬	pH4処理酸性人免疫グロブリン	人免疫グロブリンG	

別紙5

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第9回	9-1	臨床検査	C型肝炎陽性	カナダ	男	55歳	2007/1/3	未回復	症例報告	外国製品	2007/07/25、3-07000007
第7回	7-1	感染症および寄生虫症	C型肝炎	アメリカ	女	不明	不明	不明	症例報告	外国製品	2006/06/19、3-06000020
第6回	5-2	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2005/10/25、3-05000341
	6-2	感染症および寄生虫症	肝炎ウイルスキャリアー	フランス	男	不明	不明	不明	症例報告	外国製品	2005/11/15、3-05000462
第5回	5-1	臨床検査	C型肝炎陽性	カナダ	男	31歳	1998/6/4	不明	症例報告	外国製品	2005/03/25、3-04000123
	5-2	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2005/08/01、3-05000341
第4回	4-1	臨床検査	C型肝炎陽性	カナダ	女	31歳	2004/5/25	不明	症例報告	外国製品	2004/11/12、3-04000085
	4-2	感染症および寄生虫症	細菌性関節炎	フランス	男	不明	2004/3	不明	症例報告	外国製品	2004/09/28、3-04000067
	4-3	臨床検査	C型肝炎陽性	カナダ	男	63歳	2001/5/17	不明	症例報告	外国製品	2004/09/10、3-04000064
	3-35	臨床検査	C型肝炎陽性	カナダ	女	68歳	2001/3/27	不明	症例報告	外国製品	2004/09/14、3-04000057
	4-5	臨床検査	C型肝炎陽性	カナダ	女	47歳	2000/2/25	不明	症例報告	外国製品	2004/10/05、3-04000071
	4-6	臨床検査	C型肝炎陽性	カナダ	女	65歳	1999/3/11	不明	症例報告	外国製品	2004/10/06、3-04000070
	3-6	臨床検査	C型肝炎陽性	カナダ	男	72歳	1998/7/13	不明	症例報告	外国製品	2004/08/31、3-04000056
	4-8	臨床検査	C型肝炎陽性	カナダ	男	58歳	1998/6/2	不明	症例報告	外国製品	2004/09/17、3-04000063
	3-8	臨床検査	C型肝炎陽性	カナダ	女	34歳	1996/10/16	不明	症例報告	外国製品	2004/08/31、3-04000042
	3-34	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000054
	3-36	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000058
	4-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/09/17、3-04000061
	4-13	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/09/10、3-04000062
第3回	3-1	臨床検査	C型肝炎陽性	カナダ	男	69歳	2004/1/13	不明	症例報告	外国製品	2004/04/27、3-04000005
	3-2	臨床検査	C型肝炎陽性	カナダ	男	43歳	2003/8/20	不明	症例報告	外国製品	2004/04/20、3-04000004
	2-2	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/08/05、3-03000002
	3-4	臨床検査	C型肝炎陽性	カナダ	男	57歳	1999/11/9	不明	症例報告	外国製品	2004/05/10、3-04000008
	3-5	臨床検査	C型肝炎陽性	カナダ	男	84歳	1999/3/16	不明	症例報告	外国製品	2004/07/29、3-04000046

別紙様式第4

別紙5

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第3回	3-6	臨床検査	C型肝炎陽性	カナダ	男	72歳	1998/7/13	不明	症例報告	外国製品	2004/08/31、3-04000056
	3-7	臨床検査	C型肝炎陽性	カナダ	男	52歳	1997/1/9	不明	症例報告	外国製品	2004/07/23、3-04000037
	3-8	臨床検査	C型肝炎陽性	カナダ	女	34歳	1996/10/16	不明	症例報告	外国製品	2004/08/31、3-04000042
	3-9	臨床検査	C型肝炎陽性	カナダ	男	80歳	1996/2/26	不明	症例報告	外国製品	2004/08/05、3-04000031
	3-10	臨床検査	C型肝炎陽性	カナダ	男	72歳	1996/1/31	不明	症例報告	外国製品	2004/08/24、3-04000045
	3-11	臨床検査	C型肝炎陽性	カナダ	女	76歳	1996/1/11	不明	症例報告	外国製品	2004/07/13、3-04000033
	2-3	臨床検査	C型肝炎陽性	カナダ	男	75歳	1995/12/13	不明	症例報告	外国製品	2004/08/03、3-03000001
	3-13	臨床検査	C型肝炎陽性	カナダ	女	21歳	1992/2/11	不明	症例報告	外国製品	2004/07/13、3-04000040
	3-14	臨床検査	C型肝炎陽性	カナダ	男	49歳	1992/2/10	不明	症例報告	外国製品	2004/05/19、3-04000010
	2-8	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/04、3-03000003
	2-10	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000023
	2-11	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000024
	2-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000025
	2-13	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/03/25、3-03000027
	3-20	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/05/19、3-04000009
	3-21	感染症および寄生虫症	C型肝炎	ドイツ	女	不明	不明	不明	症例報告	外国製品	2004/06/01、3-04000012
	3-22	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/01、3-04000024
	3-23	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/06/28、3-04000019
	3-24	臨床検査	C型肝炎陽性	カナダ	女	46歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000036
	3-25	臨床検査	C型肝炎陽性	カナダ	女	70歳	不明	不明	症例報告	外国製品	2004/07/23、3-04000035
	3-26	臨床検査	C型肝炎陽性	カナダ	男	95歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000034
	3-27	臨床検査	C型肝炎陽性	カナダ	男	68歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000041
	3-28	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/23、3-04000039
	3-29	臨床検査	C型肝炎陽性	カナダ	不明	74歳	不明	不明	症例報告	外国製品	2004/07/13、3-04000038
	3-30	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/07/28、3-04000044

別紙5

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第3回	3-31	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/24、3-04000050
	3-32	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/24、3-04000051
	3-33	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/17、3-04000053
	3-34	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000054
	3-35	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000057
	3-36	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/08/31、3-04000058
第2回	2-1	臨床検査	C型肝炎陽性	カナダ	男	88歳	2001/4/20	不明	症例報告	外国製品	2003/12/26、3-03000016
	2-2	臨床検査	C型肝炎陽性	カナダ	男	29歳	2000/2/14	不明	症例報告	外国製品	2004/01/13、3-03000002
	2-3	臨床検査	C型肝炎陽性	カナダ	男	不明	1995/12/13	不明	症例報告	外国製品	2004/01/14、3-03000001
	2-4	臨床検査	C型肝炎陽性	カナダ	女	不明	1991/9	不明	症例報告	外国製品	2003/12/26、3-03000015
	2-5	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00050
	2-6	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00049
	2-7	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/10/07、D03-00048
	2-8	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/11/13、3-03000003
	2-9	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2003/12/24、3-03000009
	2-10	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000023
	2-11	臨床検査	C型肝炎陽性	カナダ	女	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000024
	2-12	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2004/02/26、3-03000025
	2-13	臨床検査	C型肝炎陽性	カナダ	不明	不明	不明	不明	症例報告	外国製品	2004/02/25、3-03000027
	2-14	臨床検査	C型肝炎陽性	カナダ	男	不明	2003/5	不明	症例報告	外国製品	2003/09/17、D03-00045
	2-15	臨床検査	C型肝炎陽性	カナダ	男	不明	不明	不明	症例報告	外国製品	2003/09/17、D03-00046

MedDRAバージョン10.0により集計した。

第8回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

第6回症例番号5-2は，第5回症例番号5-2において報告したものの追加報告。

第4回症例番号3-35, 3-6, 3-8, 3-34, 3-36は，それぞれ第3回症例番号3-35, 3-6, 3-8, 3-34, 3-36において報告したものの追加報告。

第3回症例番号2-2, 2-3, 2-8, 2-10, 2-11, 2-12, 2-13は，それぞれ第2回症例番号2-2, 2-3, 2-8, 2-10, 2-11, 2-12, 2-13において報告したものの追加報告。

第1回調査期間中に当該生物由来製品によると疑われる感染症の報告はなかった(初回および追加報告ともに)。

70127	2007/10/24	70646	バイエル薬品	①人血清アルブミン ②オクトコグ　アルファ(遺伝子組換え) ③インターフェロンベータ-1b(遺伝子組換え)	人血清アルブミン

別紙様式第4

別紙(5)

感染症発生症例一覧

	番号	感染症の種類		発生国	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9回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 提出日2007年7月24日
	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	1	臨床検査	C型肝炎抗体陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年7月26日
	1	感染症および寄生虫症	C型肝炎	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年8月6日
	1	臨床検査	C型肝炎抗体陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年8月6日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	男	50	2007/6/18	不明	症例報告	外国製品	識別番号3-07000010 追加報告提出日2007年8月6日
第8回		報告なし									

70130	2007/10/24	70649	CSLベーリング	フィブリノゲン加第XIII因子 人血液凝固第XIII因子	人血液凝固 第XIII因子

別紙様式第4

別紙(5)

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第9回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000033 (2007年2月23日提出の報告症例の完了報告) 完了報告書提出日2007年3月30日
	1	臨床検査	C型肝炎陽性	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000033 (2007年2月23日提出の報告症例の完了報告) 完了報告書提出日2007年3月30日
	2	感染症および寄生虫症	C型肝炎	ドイツ	男	不明	不明	不明	症例報告	外国製品	識別番号3-07000012 提出日2007年8月15日
	2	感染症および寄生虫症	B型肝炎	ドイツ	男	不明	不明	不明	症例報告	外国製品	識別番号3-07000012 提出日2007年8月15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年1月	不明	症例報告	外国製品	識別番号3-06000033　提出日2007年2月23日
	2	感染症および寄生虫症	C型肝炎	ドイツ	男	13	不明	不明	症例報告	外国製品	識別番号3-06000031　提出日2007年2月21日
	2	感染症および寄生虫症	B型肝炎	ドイツ	男	13	不明	不明	症例報告	外国製品	識別番号3-06000031　提出日2007年2月21日
	2	臨床検査	ウイルス負荷	ドイツ	男	13	不明	不明	症例報告	外国製品	識別番号3-06000031　提出日2007年2月21日

70135	2007/10/24	70654	CSLベーリング	人免疫グロブリン	人免疫グロブリン

別紙様式第4

感染症発生症例一覧

番号	感染症の種類 器官別大分類	感染症の種類 基本語	発現国	性別	年齢	発現時期	転帰	出典	区分	備考
1	感染症および寄生虫症	C型肝炎	日本	男	64	2007/7/27	③未回復	症例報告	当該製品	2007/8/28提出、識別番号1-07000082　未完了報告
2	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30提出、識別番号1-07000029　未完了報告
3	感染症および寄生虫症	C型肝炎	日本	男	42	2007/4/27	③未回復	症例報告	当該製品	2007/5/21提出、識別番号1-07000023　未完了報告
4	感染症および寄生虫症	C型肝炎	日本	男	42	2007/4/27	②軽快	症例報告	当該製品	2007/8/7提出、識別番号1-07000023　取り下げ（3番と同一症例）
5	感染症および寄生虫症	B型肝炎	日本	男	38	2007/4/17	③未回復	症例報告	当該製品	2007/4/26提出、識別番号1-07000012　未完了報告
6	感染症および寄生虫症	B型肝炎	日本	男	38	2007/4/17	③未回復	症例報告	当該製品	2007/5/10提出、識別番号1-07000012　未完了報告（5番と同一症例）
7	感染症および寄生虫症	C型肝炎	日本	男	59	2007/4/10	⑥不明	症例報告	当該製品	2007/6/15提出、識別番号1-07000040　未完了報告
8	感染症および寄生虫症	B型肝炎	日本	女	68	2007/3/29	③未回復	症例報告	当該製品	2007/4/20提出、識別番号1-07000010　未完了報告
9	感染症および寄生虫症	B型肝炎	日本	男	60	2007/3/27	②軽快	症例報告	当該製品	2007/4/13提出、識別番号1-07000007　未完了報告
10	感染症および寄生虫症	B型肝炎	日本	男	60	2007/3/27	②軽快	症例報告	当該製品	2007/4/26提出、識別番号1-07000007　未完了報告（9番と同一症例）
11	感染症および寄生虫症	敗血症性ショック	日本	男	72	2007/3/23	⑤死亡	症例報告	当該製品	2007/4/20提出、識別番号1-07000011　未完了報告
12	感染症および寄生虫症	細菌感染	日本	女	72	2007/3/9	⑤死亡	症例報告	当該製品	2007/3/27提出、識別番号1-06000272　未完了報告
13	感染症および寄生虫症	C型肝炎	日本	男	73	2007/2/23	④回復したが後遺症あり	症例報告	当該製品	2007/5/21提出、識別番号1-07000024　未完了報告
14	感染症および寄生虫症	A型肝炎	日本	女	4	2007/2/14	②軽快	症例報告	当該製品	2007/3/15提出、識別番号1-06000249　未完了報告
15	感染症および寄生虫症	C型肝炎	日本	男	66	2007/1/23	⑥不明	症例報告	当該製品	2007/6/11提出、識別番号1-07000034　未完了報告
16	感染症および寄生虫症	B型肝炎	日本	男	52	2007/1/19	③未回復	症例報告	当該製品	2007/3/6提出、識別番号1-06000236　未完了報告
17	感染症および寄生虫症	B型肝炎	日本	女	79	2006/12/13	①回復	症例報告	当該製品	2007/3/30提出、識別番号1-06000203　取り下げ 第9回症例番号17は前回報告における第8回症例番号3において報告したものの追加報告
18	感染症および寄生虫症	ＨＩＶ感染	日本	男	44	2006/3/30	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-06000009　完了報告 第9回症例番号18は前々回報告における第7回症例番号15において報告したものの追加報告
19	感染症および寄生虫症	B型肝炎	日本	女	63	2005/11/30	⑥不明	症例報告	当該製品	2007/8/30提出、識別番号1-05000224　完了報告 第9回症例番号19は前々々回報告における第6回症例番号12において報告したものの追加報告
20	感染症および寄生虫症	B型肝炎	日本	男	79	2005/9/28	①回復	症例報告	当該製品	2007/5/10提出、識別番号1-05000189　完了報告 第9回症例番号20は前々々回報告における第6回症例番号14において報告したものの追加報告
21	感染症および寄生虫症	C型肝炎	日本	男	53	2005/9/13	②軽快	症例報告	当該製品	2007/7/23提出、識別番号1-05000163　完了報告 第9回症例番号21は前々々回報告における第6回症例番号16において報告したものの追加報告
22	感染症および寄生虫症	C型肝炎	日本	女	52	2005/8/19	②軽快	症例報告	当該製品	2007/7/3提出、識別番号1-05000146　完了報告 第9回症例番号22は前々々回報告における第6回症例番号17において報告したものの追加報告

別紙様式第4

感染症発生症例一覧

第9回	23	感染症および寄生虫症	B型肝炎	日本	男	28	2005/7/15	②軽快	症例報告	当該製品	2007/5/22提出、識別番号1-05000114　完了報告 第9回症例番号23は前々々々回報告における第5回症例番号3において報告したものの追加報告
	24	感染症および寄生虫症	C型肝炎	日本	女	77	2005/7/14	①回復	症例報告	当該製品	2007/7/5提出、識別番号1-05000157　完了報告 第9回症例番号24は前々々回報告における第6回症例番号20において報告したものの追加報告
	25	感染症および寄生虫症	B型肝炎	日本	男	46	2005/5/24	⑥不明	症例報告	当該製品	2007/7/3提出、識別番号1-05000056　完了報告 第9回症例番号25は前々々々回報告における第5回症例番号8において報告したものの追加報告
	26	感染症および寄生虫症	B型肝炎	日本	男	70	2005/5/18	①回復	症例報告	当該製品	2007/8/10提出、識別番号1-05000105　完了報告 第9回症例番号26は前々々々回報告における第5回症例番号10において報告したものの追加報告
	27	感染症および寄生虫症	C型肝炎	日本	男	47	2005/5/10	③未回復	症例報告	当該製品	2007/7/3提出、識別番号1-05000149　完了報告 第9回症例番号27は前々々回報告における第6回症例番号22において報告したものの追加報告
	28	感染症および寄生虫症	B型肝炎	日本	男	73	2005/5/7	⑥不明	症例報告	当該製品	2007/6/8提出、識別番号1-05000037　完了報告 第9回症例番号28は前々々々回報告における第5回症例番号13において報告したものの追加報告
	29	感染症および寄生虫症	B型肝炎	日本	男	53	2005/4/21	⑤死亡	症例報告	当該製品	2007/5/24提出、識別番号1-05000075　完了報告 第9回症例番号29は前々々々回報告における第5回症例番号14において報告したものの追加報告
	30	感染症および寄生虫症	C型肝炎	日本	女	36	2005/4/20	①回復	症例報告	当該製品	2007/7/13提出、識別番号1-05000050　完了報告 第9回症例番号30は前々々々回報告における第5回症例番号15において報告したものの追加報告
	31	感染症および寄生虫症	B型肝炎	日本	男	69	2005/4/12	⑤死亡	症例報告	当該製品	2007/5/24提出、識別番号1-05000021　完了報告 第9回症例番号31は前々々々回報告における第5回症例番号17において報告したものの追加報告
	32	感染症および寄生虫症	B型肝炎	日本	女	53	2005/4/7	⑥不明	症例報告	当該製品	2007/6/8提出、識別番号1-05000025　完了報告 第9回症例番号32は前々々々回報告における第5回症例番号19において報告したものの追加報告
	33	感染症および寄生虫症	C型肝炎	日本	男	51	2005/2/15	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000329　完了報告 第9回症例番号33は前々々々回報告における第5回症例番号31において報告したものの追加報告
	34	感染症および寄生虫症	C型肝炎	日本	女	3	2005/1/19	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000313　完了報告
	35	感染症および寄生虫症	B型肝炎	日本	男	3	2005/1/12	③未回復	症例報告	当該製品	2007/7/11提出、識別番号1-04000311　完了報告
	36	感染症および寄生虫症	B型肝炎	日本	男	22	2005/1/8	①回復	症例報告	当該製品	2007/5/23提出、識別番号1-04000296　完了報告 第9回症例番号36は前々々々々回報告における第4回症例番号3において報告したものの追加報告

別紙様式第4

感染症発生症例一覧

	37	感染症および寄生虫症	C型肝炎	日本	男	84	2004/12/6	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000315　完了報告 第9回症例番号37は前々々々々回報告における第4回症例番号7において報告したものの追加報告
	38	感染症および寄生虫症	B型肝炎	日本	男	36	2004/11/24	①回復	症例報告	当該製品	2007/6/28提出、識別番号1-04000291　完了報告 第9回症例番号38は前々々々々回報告における第4回症例番号8において報告したものの追加報告
	39	感染症および寄生虫症	ヘルペスウイルス肺炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号39は前々々々々回報告における第4回症例番号13において報告したものの追加報告
	40	感染症および寄生虫症	ウイルス性肺臓炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号40は前々々々々回報告における第4回症例番号14において報告したものの追加報告
	41	感染症および寄生虫症	B型肝炎	日本	男	78	2004/1/19	①回復	症例報告	当該製品	2007/7/13提出、識別番号1-05000087　完了報告 第9回症例番号41は前々々々回報告における第5回症例番号41において報告したものの追加報告
	42	感染症および寄生虫症	B型肝炎	日本	女	72	2004/1/6	⑥不明	症例報告	当該製品	2007/3/29提出、識別番号1-03000103　完了報告 第9回症例番号42は前々々回報告における第6回症例番号46において報告したものの追加報告
第8回	3	感染症および寄生虫症	B型肝炎	日本	女	79	2006/12/13	⑥不明	症例報告	当該製品	2006/12/28 提出、識別番号 1-06000203 未完了報告
第7回	15	感染症および寄生虫症	ＨＩＶ感染	日本	男	44	2006/3/30	③未回復	症例報告	当該製品	2006/4/24 提出、識別番号 1-06000009 未完了報告
第6回	12	感染症および寄生虫症	B型肝炎	日本	女	63	2005/11/30	⑥不明	症例報告	当該製品	2005/12/27 提出、識別番号 1-05000224 未完了報告
	14	感染症および寄生虫症	B型肝炎	日本	男	79	2005/9/28	②軽快	症例報告	当該製品	2005/11/9 提出、識別番号 1-05000189 未完了報告
	16	感染症および寄生虫症	C型肝炎	日本	男	53	2005/9/13	②軽快	症例報告	当該製品	2005/10/5 提出、識別番号 1-05000163 未完了報告
	17	感染症および寄生虫症	C型肝炎	日本	女	52	2005/8/19	②軽快	症例報告	当該製品	2005/9/7 提出、識別番号 1-05000146 未完了報告
	20	感染症および寄生虫症	C型肝炎	日本	女	77	2005/7/14	①回復	症例報告	当該製品	2005/9/26 提出、識別番号 1-05000157 未完了報告
	22	感染症および寄生虫症	C型肝炎	日本	男	47	2005/5/10	③未回復	症例報告	当該製品	2005/9/16 提出、識別番号 1-05000149 未完了報告
	46	感染症および寄生虫症	B型肝炎	日本	女	72	2004/1/6	③未回復	症例報告	当該製品	2005/11/2 提出、識別番号 1-03000103 完了報告
第5回	3	感染症および寄生虫症	B型肝炎	日本	男	28	2005/07/15	①回復又は軽快	症例報告	当該製品	2005/08/09 提出、識別番号 1-05000114 未完了報告
	8	感染症および寄生虫症	B型肝炎	日本	男	46	2005/05/24	⑧不明	症例報告	当該製品	2005/06/20 提出、識別番号 1-05000056 未完了報告
	10	感染症および寄生虫症	B型肝炎	日本	男	70	2005/05/18	①回復又は軽快	症例報告	当該製品	2005/08/04 提出、識別番号 1-05000105 未完了報告
	13	感染症および寄生虫症	B型肝炎	日本	男	73	2005/05/07	⑧不明	症例報告	当該製品	2005/05/25 提出、識別番号 1-05000037 未完了報告
	14	感染症および寄生虫症	B型肝炎	日本	男	53	2005/04/21	死亡	症例報告	当該製品	2005/07/06 提出、識別番号 1-05000075 未完了報告
	15	感染症および寄生虫症	C型肝炎	日本	女	36	2005/04/20	①回復又は軽快	症例報告	当該製品	2005/06/17 提出、識別番号 1-05000050 未完了報告
	17	感染症および寄生虫症	B型肝炎	日本	男	69	2005/04/12	③未回復	症例報告	当該製品	2005/04/28 提出、識別番号 1-05000021 未完了報告
	19	感染症および寄生虫症	B型肝炎	日本	女	53	2005/04/07	③未回復	症例報告	当該製品	2005/05/06 提出、識別番号 1-05000025 未完了報告
	31	感染症および寄生虫症	C型肝炎	日本	男	51	2005/02/15	③未回復	症例報告	当該製品	2005/03/08 提出、識別番号 1-04000329 未完了報告
	41	感染症および寄生虫症	B型肝炎	日本	男	78	2004/01/19	③未回復	症例報告	当該製品	2005/07/25 提出、識別番号 1-05000087 未完了報告

別紙様式第4

感染症発生症例一覧

第4回	3	感染症および寄生虫症	B型肝炎	日本	男	22	2005/1/8	③未回復	症例報告	当該製品	2005/1/26 提出、識別番号 1-04000296 未完了報告
	7	感染症および寄生虫症	C型肝炎	日本	男	84	2004/12/6	③未回復	症例報告	当該製品	2005/2/18 提出、識別番号 1-04000315 未完了報告
	8	感染症および寄生虫症	B型肝炎	日本	男	36	2004/11/24	③未回復	症例報告	当該製品	2005/1/19 提出、識別番号 1-04000291 未完了報告
	13	感染症および寄生虫症	ヘルペスウイルス肺炎	日本	男	77	2004/10/14	③未回復	症例報告	当該製品	2004/11/26 提出、識別番号 1-04000244 未完了報告
	14	感染症および寄生虫症	ヘルペスウイルス胸膜炎	日本	男	77	2004/10/14	③未回復	症例報告	当該製品	2004/11/26 提出、識別番号 1-04000244 未完了報告

70143	2007/10/26	70679	日本赤十字社	新鮮凍結人血漿	新鮮凍結人血漿

別紙様式第4

感染症発生症例一覧

番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	感染症および寄生虫症	敗血症	日本	女	41	2007/7/25	③未回復	症例報告	当該製品	2007/8/16提出、識別番号1-07000075　未完了報告
2	感染症および寄生虫症	B型肝炎	日本	男	72	2007/7/11	③未回復	症例報告	当該製品	2007/9/3提出、識別番号1-07000084　未完了報告
3	感染症および寄生虫症	B型肝炎	日本	女	52	2007/7/9	③未回復	症例報告	当該製品	2007/8/16提出、識別番号1-07000078　未完了報告
4	感染症および寄生虫症	B型肝炎	日本	男	58	2007/6/25	③未回復	症例報告	当該製品	2007/8/9提出、識別番号1-07000074　未完了報告
5	感染症および寄生虫症	B型肝炎	日本	女	60	2007/6/20	②軽快	症例報告	当該製品	2007/8/9提出、識別番号1-07000073　未完了報告
6	感染症および寄生虫症	B型肝炎	日本	女	64	2007/6/13	③未回復	症例報告	当該製品	2007/7/5提出、識別番号1-07000055　未完了報告
7	感染症および寄生虫症	B型肝炎	日本	女	58	2007/6/7	②軽快	症例報告	当該製品	2007/7/5提出、識別番号1-07000054　未完了報告
8	感染症および寄生虫症	B型肝炎	日本	女	17	2007/6/6	③未回復	症例報告	当該製品	2007/7/4提出、識別番号1-07000051　未完了報告
9	感染症および寄生虫症	B型肝炎	日本	男	54	2007/6/1	③未回復	症例報告	当該製品	2007/6/18提出、識別番号1-07000042　未完了報告
10	感染症および寄生虫症	細菌感染	日本	女	66	2007/5/31	①回復	症例報告	当該製品	2007/6/18提出、識別番号1-07000041　未完了報告
11	感染症および寄生虫症	C型肝炎	日本	男	58	2007/5/31	③未回復	症例報告	当該製品	2007/6/15提出、識別番号1-07000039　未完了報告
12	感染症および寄生虫症	C型肝炎	日本	男	58	2007/5/31	③未回復	症例報告	当該製品	2007/6/15提出、識別番号1-07000039　未完了報告（11番と同一症例）
13	感染症および寄生虫症	B型肝炎	日本	男	76	2007/5/30	③未回復	症例報告	当該製品	2007/6/18提出、識別番号1-07000043　未完了報告
14	感染症および寄生虫症	B型肝炎	日本	男	76	2007/5/30	③未回復	症例報告	当該製品	2007/8/9提出、識別番号1-07000043　取り下げ（13番と同一症例）
15	感染症および寄生虫症	B型肝炎	日本	男	73	2007/5/16	⑥不明	症例報告	当該製品	2007/5/30提出、識別番号1-07000029　未完了報告
16	感染症および寄生虫症	細菌感染	日本	女	76	2007/4/26	①回復	症例報告	当該製品	2007/6/11提出、識別番号1-07000033　未完了報告
17	感染症および寄生虫症	B型肝炎	日本	男	38	2007/4/17	③未回復	症例報告	当該製品	2007/4/26提出、識別番号1-07000012　未完了報告
18	感染症および寄生虫症	B型肝炎	日本	男	38	2007/4/17	③未回復	症例報告	当該製品	2007/5/10提出、識別番号1-07000012　未完了報告（18番と同一症例）
19	感染症および寄生虫症	B型肝炎	日本	女	68	2007/3/29	③未回復	症例報告	当該製品	2007/4/20提出、識別番号1-07000010　未完了報告
20	感染症および寄生虫症	B型肝炎	日本	男	70	2007/3/28	③未回復	症例報告	当該製品	2007/6/6提出、識別番号1-07000031　未完了報告
21	感染症および寄生虫症	C型肝炎	日本	男	68	2007/3/13	③未回復	症例報告	当該製品	2007/4/2提出、識別番号1-07000002　未完了報告
22	感染症および寄生虫症	細菌感染	日本	女	72	2007/3/9	⑤死亡	症例報告	当該製品	2007/3/27提出、識別番号1-06000272　未完了報告
23	感染症および寄生虫症	B型肝炎	日本	男	65	2007/3/9	③未回復	症例報告	当該製品	2007/4/4提出、識別番号1-07000003　未完了報告
24	感染症および寄生虫症	細菌感染	日本	女	28	2007/3/2	①回復	症例報告	当該製品	2007/3/19提出、識別番号1-06000257　未完了報告
25	感染症および寄生虫症	細菌感染	日本	女	66	2007/3/1	①回復	症例報告	当該製品	2007/3/15提出、識別番号1-06000251　未完了報告
26	感染症および寄生虫症	B型肝炎	日本	女	84	2007/2/23	⑥不明	症例報告	当該製品	2007/3/27提出、識別番号1-06000271　未完了報告
27	感染症および寄生虫症	B型肝炎	日本	男	57	2007/2/21	③未回復	症例報告	当該製品	2007/3/9提出、識別番号1-06000241　未完了報告
28	感染症および寄生虫症	C型肝炎	日本	男	57	2007/2/19	①回復	症例報告	当該製品	2007/8/31提出、識別番号1-07000083　未完了報告
29	感染症および寄生虫症	B型肝炎	日本	男	15	2007/2/9	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-06000273　未完了報告
30	感染症および寄生虫症	B型肝炎	日本	女	63	2007/2/8	③未回復	症例報告	当該製品	2007/3/20提出、識別番号1-06000261　未完了報告
31	感染症および寄生虫症	B型肝炎	日本	男	64	2006/12/15	⑤死亡	症例報告	当該製品	2007/3/30提出、識別番号1-06000207　取り下げ 第9回症例番号31は前回報告における第8回症例番号7において報告したものの追加報告
32	感染症および寄生虫症	B型肝炎	日本	男	72	2006/11/4	③未回復	症例報告	当該製品	2007/3/27提出、識別番号1-06000270　未完了報告

MedDRA/J Ver. 10.0J

別紙5

別紙様式第4

感染症発生症例一覧

33	感染症および寄生虫症	B型肝炎	日本	男	72	2006/11/4	③未回復	症例報告	当該製品	2007/4/17提出、識別番号1-06000270　未完了報告
34	感染症および寄生虫症	B型肝炎	日本	男	61	2006/9/25	②軽快	症例報告	当該製品	2007/6/28提出、識別番号1-07000046　未完了報告
35	感染症および寄生虫症	B型肝炎	日本	男	73	2006/5/9	⑤死亡	症例報告	当該製品	2007/5/16提出、識別番号1-06000054　完了報告 第9回症例番号35は前々回報告における第7回症例番号15において報告したものの追加報告
36	感染症および寄生虫症	敗血症	日本	男	70	2006/5/1	⑤死亡	症例報告	当該製品	2007/4/3提出、識別番号1-06000023　完了報告 第9回症例番号35は前々回報告における第7回症例番号18及び前回報告における第8回症例番号33において報告したものの追加報告
37	感染症および寄生虫症	B型肝炎	日本	男	70	2005/12/9	⑤死亡	症例報告	当該製品	2007/5/16提出、識別番号1-06000070　完了報告 第9回症例番号37は前々回報告における第7回症例番号40において報告したものの追加報告
38	感染症および寄生虫症	B型肝炎	日本	男	28	2005/11/30	②軽快	症例報告	当該製品	2007/5/17提出、識別番号1-05000221　完了報告 第9回症例番号38は前々々回報告における第6回症例番号16において報告したものの追加報告
39	感染症および寄生虫症	B型肝炎	日本	女	63	2005/11/30	⑥不明	症例報告	当該製品	2007/8/30提出、識別番号1-05000224　完了報告 第9回症例番号39は前々々回報告における第6回症例番号17において報告したものの追加報告
40	感染症および寄生虫症	B型肝炎	日本	男	61	2005/11/17	②軽快	症例報告	当該製品	2007/8/21提出、識別番号1-05000220　完了報告 第9回症例番号40は前々々回報告における第6回症例番号18において報告したものの追加報告
41	感染症および寄生虫症	B型肝炎	日本	男	68	2005/11/11	⑥不明	症例報告	当該製品	2007/8/10提出、識別番号1-05000206　完了報告 第9回症例番号41は前々々回報告における第6回症例番号20において報告したものの追加報告
42	感染症および寄生虫症	桿菌感染	日本	男	49	2005/10/22	②軽快	症例報告	当該製品	2007/6/21提出、識別番号1-05000195　完了報告 第9回症例番号42は前々々回報告における第6回症例番号23において報告したものの追加報告
43	感染症および寄生虫症	C型肝炎	日本	男	80	2005/10/13	②軽快	症例報告	当該製品	2007/8/10提出、識別番号1-05000185　完了報告 第9回症例番号43は前々々回報告における第6回症例番号24において報告したものの追加報告
44	感染症および寄生虫症	B型肝炎	日本	男	79	2005/9/28	①回復	症例報告	当該製品	2007/5/10提出、識別番号1-05000189　完了報告 第9回症例番号44は前々々回報告における第6回症例番号28において報告したものの追加報告
45	感染症および寄生虫症	B型肝炎	日本	男	75	2005/9/20	②軽快	症例報告	当該製品	2007/7/23提出、識別番号1-05000182　完了報告 第9回症例番号45は前々々回報告における第6回症例番号31において報告したものの追加報告

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第9回	46	感染症および寄生虫症	B型肝炎	日本	男	1	2005/9/2	⑥不明	症例報告	当該製品	2007/7/5提出、識別番号1-05000152　完了報告 第9回症例番号46は前々々回報告における第6回症例番号33において報告したものの追加報告
	47	感染症および寄生虫症	細菌感染	日本	女	72	2005/9/1	①回復	症例報告	当該製品	2007/8/29提出、識別番号1-05000148　完了報告 第9回症例番号47は前々々回報告における第6回症例番号34において報告したものの追加報告
	48	感染症および寄生虫症	C型肝炎	日本	女	52	2005/8/19	②軽快	症例報告	当該製品	2007/7/3提出、識別番号1-05000146　完了報告 第9回症例番号48は前々々回報告における第6回症例番号37において報告したものの追加報告
	49	感染症および寄生虫症	C型肝炎	日本	男	66	2005/8/5	⑥不明	症例報告	当該製品	2007/6/8提出、識別番号1-05000142　完了報告 第9回症例番号49は前々々回報告における第6回症例番号39において報告したものの追加報告
	50	感染症および寄生虫症	B型肝炎	日本	男	80	2005/8/3	③未回復	症例報告	当該製品	2007/6/28提出、識別番号1-05000153　完了報告 第9回症例番号50は前々々回報告における第6回症例番号40において報告したものの追加報告
	51	感染症および寄生虫症	B型肝炎	日本	男	80	2005/8/3	③未回復	症例報告	当該製品	2007/8/30提出、識別番号1-05000153　完了報告 (50番と同一症例) 第9回症例番号51は前々々回報告における第6回症例番号40において報告したものの追加報告
	52	感染症および寄生虫症	菌血症	日本	女	77	2005/7/21	⑤死亡	症例報告	当該製品	2007/6/21提出、識別番号1-05000110　完了報告 第9回症例番号52は前々々々回報告における第5回症例番号4において報告したものの追加報告
	53	感染症および寄生虫症	B型肝炎	日本	女	4	2005/7/6	②軽快	症例報告	当該製品	2007/5/24提出、識別番号1-05000108　完了報告 第9回症例番号53は前々々々回報告における第5回症例番号6において報告したものの追加報告
	54	感染症および寄生虫症	B型肝炎	日本	女	78	2005/6/29	③未回復	症例報告	当該製品	2007/7/13提出、識別番号1-05000100　完了報告 第9回症例番号54は前々々々回報告における第5回症例番号8において報告したものの追加報告
	55	感染症および寄生虫症	B型肝炎	日本	男	5	2005/6/10	⑥不明	症例報告	当該製品	2007/3/30提出、識別番号1-05000073　完了報告 第9回症例番号55は前々々々回報告における第5回症例番号9において報告したものの追加報告
	56	感染症および寄生虫症	B型肝炎	日本	男	62	2005/5/27	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000051　完了報告 第9回症例番号56は前々々々回報告における第5回症例番号12において報告したものの追加報告
	57	感染症および寄生虫症	B型肝炎	日本	男	46	2005/5/24	⑥不明	症例報告	当該製品	2007/7/3提出、識別番号1-05000056　完了報告 第9回症例番号57は前々々々回報告における第5回症例番号13において報告したものの追加報告

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58	感染症および寄生虫症	B型肝炎	日本	女	23	2005/5/19	①回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000055　完了報告 第9回症例番号58は前々々々回報告における第5回症例番号14において報告したものの追加報告
59	感染症および寄生虫症	B型肝炎	日本	男	70	2005/5/18	①回復	症例報告	当該製品	2007/8/10提出、識別番号1-05000105　完了報告 第9回症例番号59は前々々々回報告における第5回症例番号16において報告したものの追加報告
60	感染症および寄生虫症	C型肝炎	日本	男	47	2005/5/10	③未回復	症例報告	当該製品	2007/7/3提出、識別番号1-05000149　完了報告 第9回症例番号60は前々々回報告における第6回症例番号48において報告したものの追加報告
61	感染症および寄生虫症	B型肝炎	日本	男	73	2005/5/7	⑥不明	症例報告	当該製品	2007/6/8提出、識別番号1-05000037　完了報告 第9回症例番号61は前々々々回報告における第5回症例番号18において報告したものの追加報告
62	感染症および寄生虫症	B型肝炎	日本	男	57	2005/4/26	③未回復	症例報告	当該製品	2007/6/28提出、識別番号1-05000034　完了報告 第9回症例番号62は前々々々回報告における第5回症例番号19において報告したものの追加報告
63	感染症および寄生虫症	C型肝炎	日本	男	34	2005/4/21	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000048　完了報告 第9回症例番号63は前々々々回報告における第5回症例番号20において報告したものの追加報告
64	感染症および寄生虫症	C型肝炎	日本	女	36	2005/4/20	①回復	症例報告	当該製品	2007/7/13提出、識別番号1-05000050　完了報告 第9回症例番号64は前々々々回報告における第5回症例番号21において報告したものの追加報告
65	感染症および寄生虫症	B型肝炎	日本	女	65	2005/4/15	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000060　完了報告 第9回症例番号65は前々々々回報告における第5回症例番号24において報告したものの追加報告
66	感染症および寄生虫症	B型肝炎	日本	女	59	2005/4/11	⑥不明	症例報告	当該製品	2007/8/30提出、識別番号1-05000022　完了報告 第9回症例番号66は前々々々回報告における第5回症例番号25において報告したものの追加報告
67	感染症および寄生虫症	細菌感染	日本	女	53	2005/3/2	②軽快	症例報告	当該製品	2007/7/4提出、識別番号1-04000337　完了報告 第9回症例番号67は前々々々回報告における第5回症例番号33において報告したものの追加報告
68	感染症および寄生虫症	C型肝炎	日本	男	51	2005/2/15	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000329　完了報告 第9回症例番号68は前々々々回報告における第5回症例番号40において報告したものの追加報告
69	感染症および寄生虫症	C型肝炎	日本	男	62	2005/2/10	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000331　完了報告 第9回症例番号52は前々々々回報告における第5回症例番号41において報告したものの追加報告

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70	感染症および寄生虫症	B型肝炎	日本	女	60	2005/1/31	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000328　完了報告 第9回症例番号70は前々々々回報告における第5回症例番号44において報告したものの追加報告
71	感染症および寄生虫症	B型肝炎	日本	男	67	2005/1/27	①回復	症例報告	当該製品	2007/7/9提出、識別番号1-04000348　完了報告 第9回症例番号71は前々々々回報告における第5回症例番号45において報告したものの追加報告
72	感染症および寄生虫症	B型肝炎	日本	男	65	2005/1/26	⑥不明	症例報告	当該製品	2007/7/4提出、識別番号1-04000347　完了報告 第9回症例番号72は前々々々回報告における第5回症例番号46において報告したものの追加報告
73	感染症および寄生虫症	B型肝炎	日本	男	60	2005/1/12	③未回復	症例報告	当該製品	2007/5/29提出、識別番号1-04000303　完了報告 第9回症例番号73は前々々々々回報告における第4回症例番号7において報告したものの追加報告
74	感染症および寄生虫症	B型肝炎	日本	男	53	2004/12/28	⑥不明	症例報告	当該製品	2007/6/6提出、識別番号1-04000306　完了報告 第9回症例番号74は前々々々回報告における第5回症例番号49において報告したものの追加報告
75	感染症および寄生虫症	B型肝炎	日本	女	73	2004/12/10	②軽快	症例報告	当該製品	2007/3/19提出、識別番号1-04000326　完了報告 第9回症例番号75は前々々々々回報告における第4回症例番号9において報告したものの追加報告
76	感染症および寄生虫症	C型肝炎	日本	男	84	2004/12/6	③未回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000315　完了報告 第9回症例番号76は前々々々々回報告における第4回症例番号9において報告したものの追加報告
77	感染症および寄生虫症	B型肝炎	日本	男	31	2004/11/19	⑥不明	症例報告	当該製品	2007/3/16提出、識別番号1-04000269　完了報告 第9回症例番号77は前々々々々回報告における第4回症例番号13において報告したものの追加報告
78	感染症および寄生虫症	B型肝炎	日本	男	70	2004/11/17	①回復	症例報告	当該製品	2007/3/16提出、識別番号1-04000271　完了報告 第9回症例番号78は前々々々々回報告における第4回症例番号16において報告したものの追加報告
79	感染症および寄生虫症	B型肝炎	日本	女	61	2004/11/9	⑥不明	症例報告	当該製品	2007/6/8提出、識別番号1-04000297　完了報告 第9回症例番号79は前々々々々回報告における第4回症例番号20において報告したものの追加報告
80	感染症および寄生虫症	B型肝炎	日本	男	10	2004/11/6	②軽快	症例報告	当該製品	2007/3/15提出、識別番号1-04000278　完了報告 第9回症例番号80は前々々々々回報告における第4回症例番号21において報告したものの追加報告
81	感染症および寄生虫症	ヘルペスウイルス肺炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号81は前々々々々回報告における第4回症例番号27において報告したものの追加報告

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	82	感染症および寄生虫症	ウイルス性胸膜炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号82は前々々々々回報告における第4回症例番号28において報告したものの追加報告
	83	感染症および寄生虫症	ヘルペスウイルス肺炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号83は前々々々々回報告における第4回症例番号27において報告したものの追加報告
	84	感染症および寄生虫症	ウイルス性胸膜炎	日本	男	77	2004/10/14	⑥不明	症例報告	当該製品	2007/3/15提出、識別番号1-04000244　完了報告 第9回症例番号84は前々々々々回報告における第4回症例番号28において報告したものの追加報告
	85	感染症および寄生虫症	B型肝炎	日本	男	51	2004/9/30	③未回復	症例報告	当該製品	2007/3/9提出、識別番号1-04000222　完了報告 第9回症例番号85は前々々々々回報告における第4回症例番号32において報告したものの追加報告
	86	感染症および寄生虫症	B型肝炎	日本	女	72	2004/1/6	⑥不明	症例報告	当該製品	2007/3/29提出、識別番号1-03000103　完了報告 第9回症例番号86は前々々回報告における第6回症例番号77において報告したものの追加報告
	87	感染症および寄生虫症	B型肝炎	日本	女	1	2003/1/10	②軽快	症例報告	当該製品	2007/3/29提出、識別番号1-04000072　完了報告 第9回症例番号87は前々々々々回報告における第4回症例番号30及び前々々々々々回報告における第3回症例番号30において報告したものの追加報告
第8回	7	感染症および寄生虫症	B型肝炎	日本	男	64	2006/12/15	③未回復	症例報告	当該製品	2007/1/10 提出、識別番号 1-06000207 未完了報告
	33	感染症および寄生虫症	敗血症	日本	男	70	2006/5/1	⑤死亡	症例報告	当該製品	2006/12/5 提出、識別番号 1-06000023 完了報告
第7回	15	感染症および寄生虫症	B型肝炎	日本	男	73	2006/5/9	⑤死亡	症例報告	当該製品	2006/6/22 提出、識別番号 1-06000054 未完了報告
	18	感染症および寄生虫症	敗血症	日本	男	70	2006/5/1	⑤死亡	症例報告	当該製品	2006/5/17 提出、識別番号 1-06000023 未完了報告
	40	感染症および寄生虫症	B型肝炎	日本	男	70	2005/12/9	⑤死亡	症例報告	当該製品	2006/7/18 提出、識別番号 1-06000070 未完了報告
第6回	16	感染症および寄生虫症	B型肝炎	日本	男	28	2005/11/30	⑥不明	症例報告	当該製品	2005/12/22 提出、識別番号 1-05000221 未完了報告
	17	感染症および寄生虫症	B型肝炎	日本	女	63	2005/11/30	⑥不明	症例報告	当該製品	2005/12/27 提出、識別番号 1-05000224 未完了報告
	18	感染症および寄生虫症	B型肝炎	日本	男	61	2005/11/17	②軽快	症例報告	当該製品	2005/12/22 提出、識別番号 1-05000220 未完了報告
	20	感染症および寄生虫症	B型肝炎	日本	男	68	2005/11/11	⑥不明	症例報告	当該製品	2005/12/1 提出、識別番号 1-05000206 未完了報告
	23	感染症および寄生虫症	桿菌感染	日本	男	49	2005/10/22	②軽快	症例報告	当該製品	2005/11/16 提出、識別番号 1-05000195 未完了報告
	24	感染症および寄生虫症	C型肝炎	日本	男	80	2005/10/13	⑥不明	症例報告	当該製品	2005/11/4 提出、識別番号 1-05000185 未完了報告
	28	感染症および寄生虫症	B型肝炎	日本	男	79	2005/9/28	②軽快	症例報告	当該製品	2005/11/9 提出、識別番号 1-05000189 未完了報告
	31	感染症および寄生虫症	B型肝炎	日本	男	75	2005/9/20	②軽快	症例報告	当該製品	2005/10/26 提出、識別番号 1-05000182 未完了報告
	33	感染症および寄生虫症	B型肝炎	日本	男	1	2005/9/2	⑥不明	症例報告	当該製品	2005/9/22 提出、識別番号 1-05000152 未完了報告
	35	感染症および寄生虫症	細菌感染	日本	女	72	2005/9/1	①回復	症例報告	当該製品	2005/9/16 提出、識別番号 1-05000148 未完了報告
	37	感染症および寄生虫症	C型肝炎	日本	女	52	2005/8/19	②軽快	症例報告	当該製品	2005/9/7 提出、識別番号 1-05000146 未完了報告
	39	感染症および寄生虫症	C型肝炎	日本	男	66	2005/8/5	③未回復	症例報告	当該製品	2005/9/5 提出、識別番号 1-05000142 未完了報告
	40	感染症および寄生虫症	B型肝炎	日本	男		2005/8/3	③未回復	症例報告	当該製品	2005/9/22 提出、識別番号 1-05000153 未完了報告

別紙様式第4

感染症発生症例一覧

	48	感染症および寄生虫症	C型肝炎	日本	男	47	2005/5/10	③未回復	症例報告	当該製品	2005/9/16 提出、識別番号 1-05000149 未完了報告
	77	感染症および寄生虫症	B型肝炎	日本	女	72	2004/1/6	③未回復	症例報告	当該製品	2005/11/2 提出、識別番号 1-03000103 完了報告
第5回	4	感染症および寄生虫症	菌血症	日本	女	77	2005/07/21	死亡	症例報告	当該製品	2005/08/08 提出、識別番号 1-05000110 未完了報告
	6	感染症および寄生虫症	B型肝炎	日本	女	4	2005/07/06	③未回復	症例報告	当該製品	2005/08/05 提出、識別番号 1-05000108 未完了報告
	8	感染症および寄生虫症	B型肝炎	日本	女	78	2005/06/29	⑧不明	症例報告	当該製品	2005/08/03 提出、識別番号 1-05000100 未完了報告
	9	感染症および寄生虫症	B型肝炎	日本	男	5	2005/06/10	③未回復	症例報告	当該製品	2005/07/06 提出、識別番号 1-05000073 未完了報告
	12	感染症および寄生虫症	B型肝炎	日本	男	62	2005/05/27	③未回復	症例報告	当該製品	2005/06/17 提出、識別番号 1-05000051 未完了報告
	13	感染症および寄生虫症	B型肝炎	日本	男	46	2005/05/24	⑧不明	症例報告	当該製品	2005/06/20 提出、識別番号 1-05000056 未完了報告
	14	感染症および寄生虫症	B型肝炎	日本	女	23	2005/05/19	⑧不明	症例報告	当該製品	2005/06/20 提出、識別番号 1-05000055 未完了報告
	16	感染症および寄生虫症	B型肝炎	日本	男	70	2005/05/18	①回復又は軽快	症例報告	当該製品	2005/08/04 提出、識別番号 1-05000105 未完了報告
	18	感染症および寄生虫症	B型肝炎	日本	男	73	2005/05/07	⑧不明	症例報告	当該製品	2005/05/25 提出、識別番号 1-05000037 未完了報告
	19	感染症および寄生虫症	B型肝炎	日本	男	57	2005/04/26	③未回復	症例報告	当該製品	2005/05/23 提出、識別番号 1-05000034 未完了報告
	20	感染症および寄生虫症	C型肝炎	日本	男	34	2005/04/21	③未回復	症例報告	当該製品	2005/06/14 提出、識別番号 1-05000048 未完了報告
	21	感染症および寄生虫症	C型肝炎	日本	女	36	2005/04/20	①回復又は軽快	症例報告	当該製品	2005/06/17 提出、識別番号 1-05000050 未完了報告
	24	感染症および寄生虫症	B型肝炎	日本	女	65	2005/04/15	②回復したが後遺症あり	症例報告	当該製品	2005/06/27 提出、識別番号 1-05000060 未完了報告
	25	感染症および寄生虫症	B型肝炎	日本	女	59	2005/04/11	③未回復	症例報告	当該製品	2005/04/28 提出、識別番号 1-05000022 未完了報告
	33	感染症および寄生虫症	細菌感染	日本	女	53	2005/03/02	⑧不明	症例報告	当該製品	2005/03/18 提出、識別番号 1-04000337 未完了報告
	40	感染症および寄生虫症	C型肝炎	日本	男	51	2005/02/15	③未回復	症例報告	当該製品	2005/03/08 提出、識別番号 1-04000329 未完了報告
	41	感染症および寄生虫症	C型肝炎	日本	男	62	2005/02/10	③未回復	症例報告	当該製品	2005/03/10 提出、識別番号 1-04000331 未完了報告
	44	感染症および寄生虫症	B型肝炎	日本	女	60	2005/01/31	③未回復	症例報告	当該製品	2005/03/08 提出、識別番号 1-04000328 未完了報告
	45	感染症および寄生虫症	B型肝炎	日本	男	67	2005/01/27	①回復又は軽快	症例報告	当該製品	2005/03/29 提出、識別番号 1-04000348 未完了報告
	46	感染症および寄生虫症	B型肝炎	日本	男	65	2005/01/26	③未回復	症例報告	当該製品	2005/03/29 提出、識別番号 1-04000347 未完了報告
	49	感染症および寄生虫症	B型肝炎	日本	女	73	2004/12/10	③未回復	症例報告	当該製品	2005/03/08 提出、識別番号 1-04000326 未完了報告
第4回	4	感染症および寄生虫症	B型肝炎	日本	男	60	2005/1/12	③未回復	症例報告	当該製品	2005/2/8 提出、識別番号 1-04000303 未完了報告
	7	感染症および寄生虫症	B型肝炎	日本	男	53	2004/12/28	③未回復	症例報告	当該製品	2005/2/10 提出、識別番号 1-04000306 未完了報告
	9	感染症および寄生虫症	C型肝炎	日本	男	84	2004/12/6	③未回復	症例報告	当該製品	2005/2/18 提出、識別番号 1-04000315 未完了報告
	13	感染症および寄生虫症	B型肝炎	日本	男	31	2004/11/19	③未回復	症例報告	当該製品	2004/12/22 提出、識別番号 1-04000269 未完了報告
	16	感染症および寄生虫症	B型肝炎	日本	男	70	2004/11/17	①回復	症例報告	当該製品	2004/12/28 提出、識別番号 1-04000271 未完了報告
	20	感染症および寄生虫症	B型肝炎	日本	女	61	2004/11/9	③未回復	症例報告	当該製品	2005/1/26 提出、識別番号 1-04000297 未完了報告
	21	感染症および寄生虫症	B型肝炎	日本	男	10	2004/11/6	①軽快	症例報告	当該製品	2005/1/4 提出、識別番号 1-04000278 未完了報告
	27	感染症および寄生虫症	ヘルペスウイルス肺炎	日本	男	77	2004/10/14	③未回復	症例報告	当該製品	2004/11/26 提出、識別番号 1-04000244 未完了報告
	28	感染症および寄生虫症	ウイルス性胸膜炎	日本	男	77	2004/10/14	③未回復	症例報告	当該製品	2004/11/26 提出、識別番号 1-04000244 未完了報告
	30	感染症および寄生虫症	B型肝炎	日本	女	1	2003/1/10	①軽快	症例報告	当該製品	2005/2/3 提出、識別番号 1-04000072 未完了報告
	32	感染症および寄生虫症	B型肝炎	日本	男	51	2004/9/30	③未回復	症例報告	当該製品	2004/10/20 提出、識別番号 1-04000222 未完了報告
第3回	30	感染症および寄生虫症	B型肝炎陽性	日本	女	1	2003/1/10	③未回復	症例報告	当該製品	2004/6/8提出、識別番号1-04000072

70144	2007/10/26	70680	日本赤十字社	人血小板濃厚液	人血小板濃厚液

MedDRA/J Ver. 10.0

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9回	1	感染症および寄生虫症	細菌感染	日本	女	74	2007/7/5	①回復	症例報告	当該製品	2007/7/20 提出、識別番号 1-07000062 未完了報告

70145	2007/10/26	70681	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液

MedDRA/J Ver. 10.0J

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第9回	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年6月29日 MedDRA: Version(10.0)
	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年8月29日 2007年6月29日に報告したもの追加報告 MedDRA: Version(10.0)
	9-1	臨床検査	Ｃ型肝炎陽性	日本	男性	58歳	2007/5/31	未回復	症例報告	当該製品	識別番号：07000048 報告日：2007年9月4日 2007年6月29日に報告したもの完了報告 MedDRA: Version(10.0)
第8回	該当なし										
第7回	7-1	臨床検査	Ｃ型肝炎ウイルス	米国	女性	不明	不明	不明	症例報告	当該製品	識別番号：06000021 報告日：2006年8月1日 MedDRA: Version(9.1)
第6回	該当なし										
第5回	該当なし										

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	感染症および寄生虫症	Ｃ型肝炎	日本	男性	68歳	2004/7/6	未回復	症例報告	当該製品	識別番号：04000186 報告日：2004年12月16日 MedDRA: Version(7.1)
第３回	1-1	臨床検査	Ｃ型肝炎抗体陽性	日本	女性	78歳	2003/4/2	未回復	症例報告	当該製品	識別番号：04000023 報告日：2004年4月27日 第１回症例番号1-1において報告したもの（未完了報告）の完了報告 MedDRA : Version(7.0)
第２回	該当なし										
第１回	1-1	臨床検査	Ｃ型肝炎抗体陽性	日本	女性	78歳	2003/4/2	不明	症例報告	当該製品	登録番号：A03-51 報告日：2003年8月8日（未完了報告） MedDRA: Version(6.1)

70150	2007/10/31	70708	バクスター	人血清アルブミン	人血清アルブミン

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9回	1	臨床検査	HIV検査陽性	英国	男	28ヶ月	1983年	不明	症例報告	外国製品	識別番号3-06000035 (2007年3月9日提出の報告症例の完了報告) 完了報告提出日2007年4月24日
	1	感染症および寄生虫症	後天性免疫不全症候群	英国	男	28ヶ月	1993年	不明	症例報告	外国製品	識別番号3-06000035 (2007年3月9日提出の報告症例の完了報告) 完了報告提出日2007年4月24日
	1	感染症および寄生虫症	B型肝炎	英国	男	28ヶ月	1985年	不明	症例報告	外国製品	識別番号3-06000035 (2007年3月9日提出の報告症例の完了報告) 完了報告提出日2007年4月24日
	1	感染症および寄生虫症	C型肝炎	英国	男	28ヶ月	1994年	不明	症例報告	外国製品	識別番号3-06000035 (2007年3月9日提出の報告症例の完了報告) 完了報告提出日2007年4月24日
	1	感染症および寄生虫症	A型肝炎	英国	男	28ヶ月	1994年	不明	症例報告	外国製品	識別番号3-06000035 (2007年3月9日提出の報告症例の完了報告) 完了報告提出日2007年4月24日
第8回	1	臨床検査	HIV検査陽性	英国	男	28ヶ月	1983年	不明	症例報告	外国製品	識別番号3-06000035 提出日2007年3月9日
	1	感染症および寄生虫症	後天性免疫不全症候群	英国	男	28ヶ月	1993年	不明	症例報告	外国製品	識別番号3-06000035 提出日2007年3月9日
	1	感染症および寄生虫症	B型肝炎	英国	男	28ヶ月	1985年	不明	症例報告	外国製品	識別番号3-06000035 提出日2007年3月9日
	1	感染症および寄生虫症	C型肝炎	英国	男	28ヶ月	1994年	不明	症例報告	外国製品	識別番号3-06000035 提出日2007年3月9日
	1	感染症および寄生虫症	A型肝炎	英国	男	28ヶ月	1994年	不明	症例報告	外国製品	識別番号3-06000035 提出日2007年3月9日

70158	2007/11/21	70746	CSLベーリング	乾燥pH4処理人免疫グロブリン	人免疫グロブリンG

別紙様式第4

別紙(5)

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第10回	1	感染症および寄生虫症	C型肝炎	ドイツ	男	12	1998年	不明	症例報告	外国製品	識別番号3-07000012 (2007年8月15日提出の報告症例の完了報告) 完了報告書提出日2007年9月27日
第9回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000033 (2007年2月23日提出の報告症例の完了報告) 完了報告書提出日2007年3月30日
	1	臨床検査	C型肝炎陽性	ドイツ	女	61	2007年1月	不明	症例報告	外国製品	識別番号3-06000033 (2007年2月23日提出の報告症例の完了報告) 完了報告書提出日2007年3月30日
	2	感染症および寄生虫症	C型肝炎	ドイツ	男	不明	不明	不明	症例報告	外国製品	識別番号3-07000012 提出日2007年8月15日
	2	感染症および寄生虫症	B型肝炎	ドイツ	男	不明	不明	不明	症例報告	外国製品	識別番号3-07000012 提出日2007年8月15日

70161	2007/11/21	70749	CSLベーリング	人免疫グロブリン	人免疫グロブリン