資料3

感染症的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。

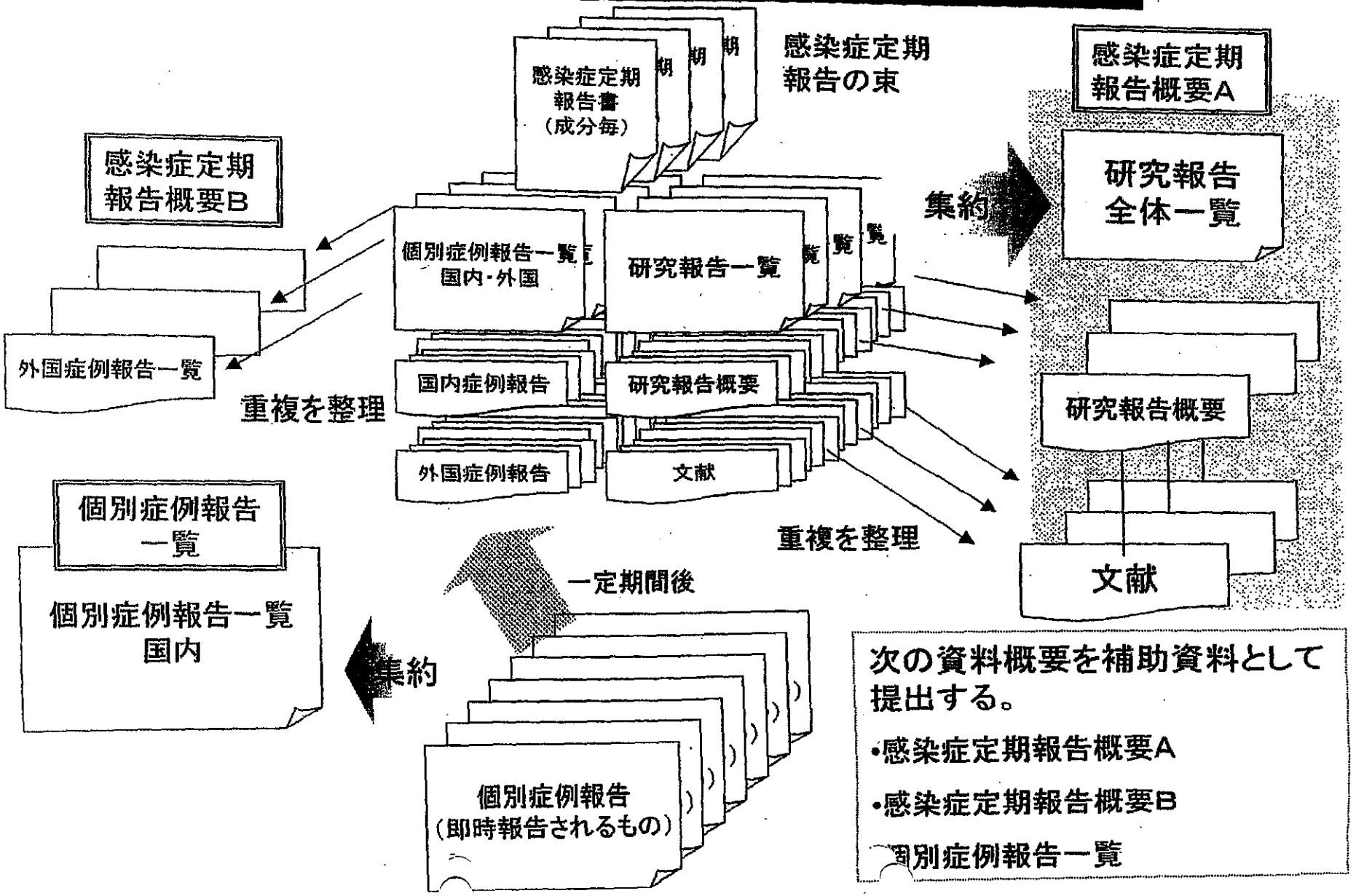
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた**「資料概要B」**を事務局が作成し、送付する。

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・感染症個別症例報告の取り扱い



次の資料概要を補助資料として提出する。

- 感染症定期報告概要A
- 感染症定期報告概要B
- 個別症例報告一覧

感染症定期報告概要

（平成１９年１１月１４日）

平成１９年６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６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(2007/6/1～2007/8/31)

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70059	2007/06/15	70257	A型肝炎	J Med Virol 2006; 78: 1398-1405	A型肝炎ウイルス(HAV)感染患者の血液および糞便中へのウイルス排泄期間および排泄量と、アラニンアミノトランスフェラーゼ(ALT)、疾患重症度、HAV遺伝子型との関連を調べた。27例の急性HAV患者でHAVは発症後81日間(中央値)便中に排泄され、半数で36日目でも多量なウイルスの排泄が続いた。ウイルス血症は検出されたが、定量できなかった(中央値42日間)。疾患発症後10日間は、ALT値が高いほど血中ウイルス量が高かった。遺伝子型1aと1bの患者で、HAV排泄および黄疸の期間に有意差はなかった。	
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	B型肝炎	Transfusion 2006; 46: 2028-2029	2004年10月、神奈川県赤十字血液センターは輸血後HBV感染疑い症例の報告を受けた。供血当時の検査では50プールNAT陰性だったにも関わらず、凍結検体がHBV個別NAT陽性となった供血者を特定した。この供血者の凍結血液40検体について個別NATを行ったところ、陰性と陽性があった。合計6例の輸血後HBV感染が特定された。この供血者におけるHBV DNAの量は50コピー/mL未満から200コピー/mLの間で増減していた。供血前に個別NATを行ったとしても、全てのHBVキャリアを排除できないことが示された。	
70059	2007/06/15	70257	B型肝炎 C型肝炎	Transfusion 2006; 46: 1997-2003	健康歴の問診によって供血延期となった供血者497名を、4つの米国赤十字血液センターで募集し、血液感染症の血清マーカーについて血液検体を検査した。その結果、ウイルス肝炎リスクおよび静注薬物使用歴に関する標準的な供血者用問診にて供血停止となった供血者は、供血停止とならなかった供血者よりも肝炎マーカー陽性率が高い場合が多かった。その他のマーカーおよび質問について有意な知見は認めなかった。	
70059	2007/06/15	70257	C型肝炎	JAMA 2006; 296: 2005-2011	2004年10月15日にメリーランドで放射性医薬品注射剤を用いて心筋灌流試験を行った患者16名に発生した急性HCV感染について調べた。患者はある薬局で調整された1つのバイアルの注射剤を投与されていた。その薬局では、注射剤を調製する12時間前に、HCVおよびHIVに罹患した患者の血液の放射線標識白血球測定を行っていた。この患者から得られたHCVのシークエンスは、当該16症例の配列とほぼ同一であった(相同性97.8%～98.5%)。生物由来製剤を取り扱う放射性医薬品薬局は、適切な無菌操作を行うべきである。	
70078	2007/07/27	70346	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。	1

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70069	2007/07/18	70317	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。	
70069	2007/07/18	70317	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。	2
70061	2007/06/15	70259	HHV-8感染	N Engl J Med 2006; 355: 1331-1338	2000年12月から2001年10月に輸血を受けたウガンダのKampalaの患者1811例のうち、輸血前にヒトヘルペスウイルス8型(HHV-8)血清陰性であった患者991例について追跡調査を行った。そのうち43%(425例)にHHV-8血清陽性血が輸血された。991例中41例にHHV-8セロコンバージョンが起こったが、セロコンバージョンのリスクは陽性血を輸血された患者の方が陰性血を輸血された患者より有意に高かった。	
70070	2007/07/24	70324	HIV	FDA/CBER 2007年3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。	3
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	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。	4
70069	2007/07/18	70317	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。	

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70069	2007/07/18	70317	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。	5
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	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。	6
70061	2007/06/15	70259	クロイツフェルト・ヤコブ病	Vox Sang 2006; 91(Suppl. 3): 68-69	米国で供血後に古典的CJDを発症した個人を特定し、受血者の追跡調査を行った。2006年2月までに、古典的CJDで死亡した供血者31名が試験に登録され、384名の受血者が特定された。追跡を行ったところ、古典的CJDが輸血を介して受血者に伝播したという証拠は示されなかった。本研究は、1995年に米国の大規模血液供給システムと疾病対策予防センター(CDC)により開始され、継続中である。	
70069	2007/07/18	70317	異型クロイツフェルト・ヤコブ病	AABB Weekly Report 2006; 12(44): 4-5	伝達性海綿状脳症(TSE)諮問委員会が2006年12月15日に公開で開催され、ヒト血漿由来抗血友病因子(FVIII)製剤におけるvCJDへの潜在的曝露に関するFDAのリスク評価ならびに血漿由来FVIII製造におけるTSEクリアランスのレベルについて討論された。このリスク評価に対して諮問委員会は、報告が強制でないことや、最終製品のリスク減少を推定をする際に用いたエビデンスに対して懸念を表明した。	
70061	2007/06/15	70259	異型クロイツフェルト・ヤコブ病	ABC Newsletter 2006年12月15日 5-6ページ	米国で製造された血漿由来の第Ⅷ因子製剤による患者へのvCJD病原体伝播のリスクは、極めて低いと見られる。生物製剤評価調査センター(CBER)のSteven Anderson博士は、「しかし、リスクはゼロではない」と伝達性海綿状脳症(TSE)諮問委員会の本日の会合で話した。CBERは、2005年10月31日の委員会で提示されたコンピュータモデルと仮説に基づいたリスク分析案の概要を示した。重要度解析では、リスクを決定する主要な要素は、製造工程におけるvCJD感染因子の低減である。	
70059	2007/06/15	70257	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。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献
70078	2007/07/27	70346	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。	7
70078	2007/07/27	70346	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。	8
70069	2007/07/18	70317	異型クロイツフェルト・ヤコブ病	CDC 2006年11月29日	米国で3例目のvCJD症例が確定された。サウジアラビアで生まれ育った若年成人で、2005年後半から米国に住んでいる。2006年11月下旬にアデノイドおよび脳生検により確定診断された。この患者に輸血歴やヨーロッパ訪問歴はなく、子供の頃にサウジアラビアでBSE感染牛製品を摂食したことが原因と思われる。この患者に供血歴はなく、公衆衛生学的調査により、米国住民への伝播の危険はないと同定された。	(
70070	2007/07/24	70324	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。	9
70059	2007/06/15	70257	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。	10 (
70064	2007/06/22	70281	異型クロイツフェルト・ヤコブ病	FDA/CBER 2006年10月15日 FDA/TSEAC Meeting 2006年12月15日	FDAは、米国で認可されたヒト血漿由来第VIII凝固因子製剤(pdFVIII)の使用に係る潜在的vCJDリスク評価草案を作成した。FDAの評価モデルの結果は、血友病Aおよびフォンウィルブランド病患者に使用されるpdFVIII製剤の、vCJD感染リスクは非常に低いが、ゼロではないかもしれないことを示唆した。またTSEAC(TSE Advisory Committee)は、pdFVIII製品中のTSE除去の適切な閾値について議論した。TSE除去レベルにより、vCJD感染リスクは大きく変動することが示された。	
70065	2007/06/29	70299	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。	

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70070	2007/07/24	70324	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。	
70070	2007/07/24	70324	異型クロイツフェルト・ヤコブ病	FDA/CBER 2007年3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。	11
70069	2007/07/18	70317	異型クロイツフェルト・ヤコブ病	HPA Press Statement 2007年1月18日	輸血と関係した新たなvCJD疾患(4例目)が、最近診断された。この症例は後にvCJDを発症したドナーからの輸血を受けてから約9年後にvCJDと診断された。同じ供血者からの輸血は以前に同定された1例とも関係していた。4例目の患者は以前からvCJDに暴露した可能性を知らされていた。4例目のvCJD感染症例により、輸血を介したヒトの間におけるvCJD感染リスクについての懸念が高まっている。4症例は全て、成分輸血に関係したものであり、血漿分画製剤による治療に関連した症例は今まで報告されていない。	
70059	2007/06/15	70257	異型クロイツフェルト・ヤコブ病	Health Protection Report 1(3) 2007年1月19日	英国で4例目の輸血関連vCJD可能性例が診断された。この症例は供血後約17ヶ月でvCJDを発症したドナーからの赤血球輸血を受け、8年半後にvCJDを呈した。このドナーは3例目の輸血関連vCJD症例へのドナーでもある。4例目の症例はプリオン蛋白遺伝子のコドン129がメチオニンホモ体であった。まだ生存中である。	
70069	2007/07/18	70317	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。	
70059	2007/06/15	70257	異型クロイツフェルト・ヤコブ病	Lancet 2006; 368: 2061–2067	vCJDを発症した供血者の輸血を受けた患者が神経学的徴候を発現し、National Prion Clinicへ照会され、vCJDと診断された後、MRC PRION-1 trialに登録された。患者が死亡した際、剖検時に脳と扁桃腺の組織を得、免疫ブロッティング法および免疫組織化学検査により異常プリオンの存在を調べた。剖検により診断が確認され、扁桃腺のプリオン感染が示された。扁桃腺の生検は、BSEプリオンの1次感染患者と同様、医原的曝露を被った他の高リスク患者においても、早期の症状発現前診断を可能にする。	
70069	2007/07/18	70317	異型クロイツフェルト・ヤコブ病	LANCET 2006; 368: 2226–2230	ヒト濃縮赤血球に混入した脳由来の感染性物質を約4 log ID50減らすことのできるアフィニティ樹脂L13と同等能力のL13Aとについて、血中に存在する内因性TSE感染性物質の除去能力を評価した。スクレイピーに感染させたハムスターの全血は白血球除去によって感染性の72%が除去された。99匹中15匹が白血球除去した全血に感染したが、更に各々の樹脂を通過して得られた最終産物を接種された96匹又は100匹はいずれも発症しなかった。樹脂によって内因性TSE感染性物質が除去されることが示された。	

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70059	2007/06/15	70257	異型クロイツフェルト・ヤコブ病	PLoS ONE 2006; 1: e71	プリオン蛋白に高親和的、特異的に結合する吸着基質Alicon Prio Trapを開発し、ヒト、ウシ、ヒツジ、ヤギの乳汁中にPrPScの前駆体であるPrPCを同定することができた。PrPCの絶対量には種差があり、ヒツジの乳汁中でμg/lレンジ、ヒト乳汁中ではng/lレンジであった。PrPCは、均質化し低温殺菌した市販ミルク中にも認められ、超高温処理を施しても内因性PrPC濃度はわずかに減少しただけであった。TSEに感染した動物の乳汁がPrPScの感染源となる可能性を示唆する。	
70064	2007/06/22	70281	異型クロイツフェルト・ヤコブ病	PLoS Pathogens 2006; 2: 956-963	最近、大規模なスクリーニングによって、従来とは異なるPrPresがウシにおいて発見された。H型と呼ばれる高分子量のフランスのウシPrPres分離株を、ウシまたはヒツジのPrPを発現するトランスジェニックマウスに接種した。全てのマウスは神経学的症状を呈し、死亡し、これらの株が感染性プリオンの新規株であることが示された。この病原体は、BSE病原体およびヒツジスクレイピー病原体とは明らかに異なる特有の神経病理学的特徴を示した。	
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	異型クロイツフェルト・ヤコブ病	ProMED-mail20070108.0081	英国保健省は2007年1月8日、CJD患者数に関する最新情報を公表した。vCJD確定例における死亡患者112名、vCJD可能性例における死亡患者(神経病理学的に未確定)46名で、死亡患者総数は158名である。生存中のvCJD可能性患者は7名で、vCJD確定例または可能性例総数は165名である。2006年12月4日の月例統計以来、死亡患者総数には変化なく、確定例または可能性例総数は1名増加した。このデータは英国におけるvCJD流行は減少しつつあるとする見解に一致する。	
70061	2007/06/15	70259	異型クロイツフェルト・ヤコブ病	Science 2006; 314: 133-136	慢性消耗病(CWD)非感染シカをCWD陽性のシカの唾液、血液または尿・糞に曝露させた。その結果、CWDを伝播しうる感染性プリオンが唾液および血液中に認められた。CWDはシカ科の動物に容易に伝播すると言える。プリオン感染では体液との接触に関する注意が払われるべきである。	
70070	2007/07/24	70324	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。	12
70061	2007/06/15	70259	異型クロイツフェルト・ヤコブ病	Vox Sanguinis 2006; 91(Suppl 3): 70	PRDT(Pathogen Removal and Diagnostics Technologies)は、全血、RBCまたは血漿存在下で,脳由来プリオンタンパク質およびTSE感染物と強く結合する高親和性リガンドを得るため、何百万もの化合物をスクリーニングした。その結果、PRDTのリード樹脂は赤血球存在下でも高濃度のTSE感染物を吸着し、低濃度の内因性TSE感染物を除去した。この樹脂を使用したMacoPharma P-Capt(TM)フィルターを用いることにより、輸血によるvCJD伝播リスクを軽減できる。	

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70069	2007/07/18	70317	アルツハイマー型認知症	Science 2006; 313: 1781-1784	アルツハイマー病患者、またはβ-アミロイド前駆体タンパク質(APP)発現トランスジェニックマウスから得たアミロイドーβ(Aβ)含有脳抽出物の希釈液を大脳内に注射すると、APPトランスジェニックマウスに、時間と濃度に依存した大脳内のβ-アミロイドーシスとそれに伴う病変を誘発した。脳抽出物のシーディング活性は、Aβ免疫除去、タンパク変性、またはAβを宿主に免疫することによって、低下または消失した。外因性に誘発させたアミロイドーシスの表現型は、宿主と誘導物質の両者に依存した。	
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	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。	
70064	2007/06/22	70281	ウイルス感染	J Infect Dis 2006; 194: 1276-1282	ヒトボカウイルス感染の疫学的プロフィールおよび臨床的特徴を調べるため、2歳未満の小児のヒトボカウイルスを調査した。直接的免疫蛍光試験でRSV(respiratory syncytial virus)、パラインフルエンザウイルス(1-3型)、インフルエンザAおよびB、並びにアデノウイルスが陰性であった425名中22名(5.2%)がPCRでヒトボカウイルス陽性であり、無症候であった96名では陽性者はゼロであった。この試験期間中、2つの異なる遺伝型が見られた。	
70059	2007/06/15	70257	ウイルス感染	ProMED-mail20061223.3593	日本でノロウイルスによる感染性胃腸炎が増加している。この疾患は従来食中毒とされてきたが、昨年の症例のうち生の貝類摂食に関連したものは15%しかなく、患者の吐瀉物や排泄物から、あるいはウイルスが手を介して食物や食器に付着することで間接的に感染することが多い。今シーズンのノロウイルス流行は主にヒトーヒト感染によるものであり、変異による新たなウイルス株の流行と考えられる。2006年11月27日から12月3日までの間に、全国の約3000の医療機関から65,638人の感染患者が報告された。	
70059	2007/06/15	70257	ウイルス感染	ProMED-mail20070106.0058	2006年12月23日、ケニアGarissaの公立病院に入院した患者複数の症例から、リフトバレー熱のヒトでのアウトブレイクが初めて確認された。IgM及びPCRにより確定診断された。同地区での発病率は、19/10万人で、最高値は最初に患者が見つかったShanta Abakの129/10万人である。2007年1月5日現在で188例に達し、うち68例が死亡した。2007年1月4日、ケニヤ北東部のIjara地区でリフトバレー熱の新規疑い例8例が発見された。	
70059	2007/06/15	70257	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。	

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70059	2007/06/15	70257	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。	
70067	2007/07/10	70314	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パ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。	13
70075	2007/07/26	70336	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。	
70059	2007/06/15	70257	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。	
70059	2007/06/15	70257	ウエストナイルウイルス	ProMED-mail20061214.3510	2006年、米国におけるウエストナイルウイルス感染のヒト症例は43州から4052例が報告され、うち1396例で脳炎や髄膜炎を発症、死亡例は146例だった。また、ウマ、トリ、蚊からのウイルス検出が報告されている。	
70059	2007/06/15	70257	ウエストナイルウイルス	Transfusion 2006; 46: 2036-2037	ウエストナイルウイルス(WNV)が輸血感染するとの認識により、米国とカナダではウイルスRNAに関する供血者の検査が迅速に導入された。最近の分析ではこの検査は費用対効果が低いと指摘されている。Custerらは、ミニプール検査と一部個別検査を組み合わせた通年の検査は、費用対効果は低いが血液安全のためには最善の選択であるとしている。一方Korvesらは、検査の削減を提唱している。検査の効率性を問う必要はあるが、WNVスクリーニングを行う他の方法がないかを検討することも重要である。	
70069	2007/07/18	70317	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。	
70082	2007/07/27	70350	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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70059	2007/06/15	70257	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。	14
70059	2007/06/15	70257	チクングニヤウイルス感染	毎日新聞 2007年1月24日	1月24日、厚生労働省はスリランカから帰国した30歳代の女性が、チクングニヤ熱に感染していたと発表した。国内で日本人の感染が確認されたのは初めてである。女性は2006年11月中旬、スリランカで発熱し、現地でチクングニヤ熱かデング熱と診断された。女性はすでに症状は回復し、在住するスリランカに戻っている。厚労省によると、チクングニヤ熱は発熱や関節炎、発疹などが特徴で、死亡率は極めて低い。蚊を介して感染し、人から人への感染はない。	
70059	2007/06/15	70257	トリパノソーマ症	AABB Weekly Report 2006; 12(43): 1-2	FDAがシャーガス病の供血者スクリーニング検査試薬を初めて認可したのを受け、米国血液銀行協会(AABB)は、採血施設が検査導入とその期間を決定し、供血者と受血者のフォローアップのためのガイダンスを提供するのに役立つよう、協会公報#06-08を12月14日に発表した。具体的な勧告内容は、出荷停止、遡及調査、自己血輸血で繰り返し検査陽性となった場合の成分製剤出荷の認可、供血延期措置、通知、確認試験、供血者の医学的評価のための供血延期などの事項が盛り込まれている。	
70069	2007/07/18	70317	トリパノソーマ症	FDA News P06-198 2006年12月13日	米国FDAは2006年12月13日、重篤且つ致死性の寄生虫感染症のシャガス病を引き起こす血液寄生虫について血液ドナーをスクリーニングする新しい検査を承認した。この試験はORTHO T. cruzi ELISA Test Systemと呼ばれ、trypanosoma cruzi抗体を検出するもので、このような検査では初めてFDAに承認されたものである。この検査は、全血の供血者のスクリーニングに加えて、臓器、細胞及び組織ドナーからの血漿及び血清をスクリーニングするのに用いられる。	
70059	2007/06/15	70257	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。	15
70059	2007/06/15	70257	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。	
70069	2007/07/18	70317	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。	16

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70069	2007/07/18	70317	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。	17
70069	2007/07/18	70317	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。	18
70059	2007/06/15	70257	マラリア	Eurosurveillance 2006年11月16日	2006年8月にコルシカ島で三日熱マラリア1症例が診断された。フランス南東部出身の59歳男性で、2006年夏に南コルシカのポルトに滞在していた。患者はマラリア流行地域への渡航歴はなかった。マダガスカルに渡航歴のある三日熱マラリア患者が7月初めに同地区に滞在しており、コルシカのハマダラカによってP. vivaxの国内伝播が起こったことを示唆している。 本症例は、この地域で報告されたマラリアの地域内伝播の1972年以来初の症例である。	
70059	2007/06/15	70257	マラリア	Eurosurveillance weekly release 2007; 12(1): 070111	輸入感染症サーベイランスに関するヨーロッパネットワークへの報告数によると、2006年11月下旬以降、インド、ゴア州への渡航者において、熱帯熱マラリア患者が増加している。1月10日までに、ドイツで2例、デンマークで4例、スエーデンで2例、計8例の患者が報告された。	
70078	2007/07/27	70346	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。	19
70069	2007/07/18	70317	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。	
70059	2007/06/15	70257	感染	Blood 2006; 108: Abstract #4144	0.2mM S-303 (アクリジン化合物) および20mM グルタチオン(GSH)を用いた改良S-303処理法を用い、RBC中の細菌およびウイルス不活化の有効性を評価した。輸血に関連するグラム陽性菌およびグラム陰性菌、Vesicular stomatisウイルス、Adenovirus 5、HIVおよびウシウイルス性下痢ウイルス(HCVのモデル)のいずれも改良S-303処理により効果的に不活化された。	

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70059	2007/06/15	70257	狂犬病	ProMED-mail20061118.3303	2006年11月17日、京都府の保健所は、京都市の60歳代の男性がフィリピンで犬にかまれ、帰国後に狂犬病を発症して死亡したと発表した。厚労省によると、日本人が国内で狂犬病を発症したのは36年ぶりである。厚労省によると、男性はフィリピン滞在中の8月末に野良犬にかまれ、11月1日に帰国した。9日に風邪のような症状で京都市内の病院を受診した。その後、幻覚症状、水や風を怖がるなど狂犬病特有の症状を発症した。国立感染症研究所が調べたところ、男性の唾液から狂犬病ウイルスが検出された。	
70069	2007/07/18	70317	結核	NIKKEI NET いきいき健康 2006年12月5日	既存の治療薬がほとんど効かず、世界保健機関(WHO)が警戒を呼び掛けている「超多剤耐性」の結核菌が、国内でも入院患者の0.5%から検出されたことが、結核研究所の調査で明らかになった。2002年6月から11月にかけて国内99の結核治療施設の入院患者3122人から採取した結核菌を分析した結果である。検出例の半数は薬の服薬歴がなかったことから、他の患者から感染した可能性が高い。	
70059	2007/06/15	70257	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。	20
70078	2007/07/27	70346	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。	21
70078	2007/07/27	70346	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。	22
70059	2007/06/15	70257	鳥インフルエンザ	Eurosurveillance 2006; 11(12): 061221	2006年11月29日時点でH5N1型トリインフルエンザウイルス感染患者258名がWHOに報告され、50カ国以上で鳥類での感染が確認されており、うち10カ国では鳥類がヒト患者発生の感染源となっている。EUでは、同ウイルスは家禽には感染定着しておらず、2006年春季に少なくとも15カ国で野鳥の感染が確認されたが、ヒト感染症例は発生していない。家禽の感染予防が成功し、感染は5件のみで迅速に制圧された。散発例の報告が続いていることから、生物学的安全確保対策と早期警報システムを堅持する必要がある。	
70059	2007/06/15	70257	鳥インフルエンザ	ProMED-mail20061201.3394	WHOは、H5N1鳥インフルエンザウイルスにより光を当て、パンデミック株への変異の検出を容易にするために、H5N1鳥インフルエンザのヒト症例調査のためのガイドラインを発表した。14ページのガイドラインは、患者の問診、周辺で他の症例を捜索することによる接触歴の調査、ヒトーヒト感染の何らかの徴候を発見するためのデータのふるいわけなど、各症例の徹底的な調査を求めている。ガイドラインでは、臨床検査の結果が出る前に疑い症例の調査を行うことを要請している。	

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70059	2007/06/15	70257	鳥インフルエンザ	ProMED-mail20070120.0260	2007年1月18日、農林水産省は、宮崎県の養鶏場で発生したトリインフルエンザは高病原性ウイルスによるものだったと明らかにした。同省は養鶏場で死亡した鶏から採取したウイルスのサンプルを検査して病原性が高いものであることを確認した。H5N1型ウイルスの流行は、宮崎県清武町の谷口孵卵場黒坂農場で発生し、3つある鶏舎のうち1つで3500羽の鶏が死亡した。	
70059	2007/06/15	70257	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、ヒトアルブミンの低温殺菌、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。	
70070	2007/07/24	70324	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。	
70059	2007/06/15	70257	梅毒	Lancet 2007; 369: 132-138	中国の性感染症サーベイランスシステム及び監視サイトネットワークからの症例報告データを収集し評価した。中国における報告された梅毒の全症例発生率は、1993年には100,000人あたり0.2例であったが、2005年には、第一期及び第二期梅毒だけで100,000人あたり5.7例であった。先天的な梅毒の発生率は、1991年は100,000例の出生児あたり0.01症例であったが、2005年には100,000例の出生児あたり19.68症例まで、年平均71.9%の割合で大きく増加した。	
70059	2007/06/15	70257	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	23

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. 5. 15	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況	Gotanda Y, Iwata A, Ohnuma H, Yoshikawa A, Mizoguchi H, Endo K, Takahashi M, Okamoto H. J Med Virol. 2007 Apr 24;79(6):734-742.	公表国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)			日本

研究報告の概要

○本邦におけるアラニンアミノトランスフェラーゼ高値供血者の無症候性E型肝炎感染の現況
E型肝炎ウイルス(HEV)の無症候性感染の現況は完全には調査されていない。本試験では、日本赤十字血液センターでアラニンアミノトランスフェラーゼ(ALT)高値(61-476 IU/L)を示した献血者のボランティア6700名から血清検体を採取し、ELISA を用いて抗HEV IgG、IgM、IgAの有無を調べ、nested RT-PCR を用いてHEV RNAを検査した。全体として、479名(7.1%)の供血者が抗HEV IgG陽性であり、そのうち8名は抗HEV IgM陽性、7名は抗HEV IgA陽性であった。抗HEV IgM および(または)抗HEV IgA陽性患者9名のうち、6名にHEV RNAが検出された。残り6691名から得た血清10検体のミニプールでさらにHEV RNAを調べたところ、3名がHEV RNA陽性であった(うち1名は抗HEV IgG陰性)。ALT値で層別化した場合、ALT≧201 IU/Lの供血者109名のHEV RNAの陽性率は、ALT 61-200 IU/Lの供血者6591名と比較して有意に高かった(2.8% vs. 0.1%, P < 0.0001)。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、85.6～98.5%の一致度があり、オープンリーディングフレーム 2の412-ヌクレオチド配列における日本固有HEV株 (JRA1)との相同性は87.3～93.9%であった。本試験は、ALT≧201 IU/Lの日本人の約3%はさまざまなHEV株の無症候性感染を有することを示している。

使用上の注意記載状況・その他参考事項等

白血球除去赤血球「日赤」
照射白血球除去赤血球「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
ALT≧201 IU/Lの日本人の約3%はさまざまなHEV株の無症候性感染を有することが示されたとの報告である。日本赤十字社では、献血血液のALT検査を行い、61 IU/L以上の血液を排除している。	日本赤十字社では、厚生労働省科学研究「本邦に於けるE型肝炎の診断・予防・疫学に関する研究班」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。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。

Journal of Medical Virology 79:734–742 (2007)

Ongoing Subclinical Infection of Hepatitis E Virus Among Blood Donors With an Elevated Alanine Aminotransferase Level in Japan

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Ongoing subclinical infection of hepatitis E virus (HEV) has not been fully studied. In the present study, serum samples were collected from 6700 voluntary blood donors with an elevated alanine aminotransferase (ALT) level of 61-476 IU/l at a Japanese Red Cross Blood Center, and were tested for the presence of IgG, IgM and IgA classes of antibodies to HEV (anti-HEV) by in-house ELISA and HEV RNA by nested RT-PCR. Overall, 479 blood donors (7.1%) were positive for anti-HEV IgG, including 8 donors with anti-HEV IgM and 7 donors with anti-HEV IgA. Among the nine donors with anti-HEV IgM and/or anti-HEV IgA, six had detectable HEV RNA. The presence of HEV RNA was further tested in 10-sample minipools of sera from the remaining 6691 donors, and three donors including one without anti-HEV IgG were found to be positive for HEV RNA. When stratified by ALT level, the prevalence of HEV RNA was significantly higher among the 109 donors with ALT ≥201 IU/l than among the 6591 donors with ALT of 61–200 IU/l (2.8% vs. 0.1%, $P<0.0001$). The HEV isolates obtained from the nine viremic donors segregated into genotype 3, shared a wide range of identities of 85.6–98.5% and were 87.3–93.9% similar to the Japan-indigenous HEV strain (JRA1), in the 412-nucleotide sequence of open reading frame 2. This study suggests that approximately 3% of Japanese individuals with ALT ≥201 IU/l have ongoing subclinical infection with various HEV strains. ***J. Med. Virol. 79: 734–742, 2007.*** © 2007 Wiley-Liss, Inc.

KEY WORDS: hepatitis E virus; subclinical infection; PCR; genotype; phylogenetic analysis

INTRODUCTION

Hepatitis E is an acute disease that is endemic in many developing countries of Asia and Africa where sanitation is suboptimal, and is also endemic in many industrialized countries including the United States, European countries and Japan [Harrison, 1999; Purcell and Emerson, 2001; Smith, 2001; Emerson and Purcell, 2003; Okamoto et al., 2003]. Hepatitis E virus (HEV), the causative agent of hepatitis E, is a positive-sense RNA virus without an envelope and is classified as the sole member of the genus *Hepevirus* in the family *Hepeviridae* [Emerson et al., 2004]. Its genome is approximately 7.2 kilobases in length and contains three open reading frames (ORFs: ORF1, ORF2 and ORF3) flanked by short untranslated regions [Tam et al., 1991]. ORF1 is the largest of the three and encodes viral non-structural proteins. ORF2 encodes the capsid protein and ORF3 encodes a small protein that undergoes phosphorylation [Koonin et al., 1992; Zafrullah et al., 1997]. Extensive genomic diversity has been noted among HEV isolates and HEV sequences have been classified into four genotypes (genotypes 1–4) [Schlauder and Mushahwar, 2001]. Genotype 1 HEV has been responsible for a number of waterborne epidemics of

The nucleotide sequence data reported in this study have been assigned DDBJ/EMBL/GenBank accession numbers AB288357–AB288365.

Grant sponsor: Ministry of Education, Culture, Sports, Science, and Technology of Japan; Grant sponsor: Ministry of Health, Labor, and Welfare of Japan.

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Accepted 7 February 2007

DOI 10.1002/jmv.20834

Published online in Wiley InterScience (www.interscience.wiley.com)

hepatitis E in Asia and Africa. Although HEV of genotype 2 has been detected less frequently, it was responsible for outbreaks in Mexico in 1986–1987 [Velazquez et al., 1990] and has been implicated in sporadic infections in Africa [Buisson et al., 2000]. On the other hand, genotypes 3 and 4 HEV cause sporadic cases of acute hepatitis but have not been found to be responsible for epidemics in humans; these infections seem to be zoonotic and both genotypes have been detected in pigs (genotype 3 worldwide, and genotype 4 in Asia), which may constitute the major reservoir of genotypes 3 and 4 [Harrison, 1999; Meng, 2005; Lu et al., 2006].

Polyphyletic HEV strains of genotypes 3 and 4 are circulating in Japan [Takahashi et al., 2001, 2002; Mizuo et al., 2002; Inoue et al., 2006] and HEV has been recognized as an important causative agent of sporadic acute hepatitis of non-A, non-B, non-C etiology in this country [Mizuo et al., 2002; Okamoto et al., 2003]. It has been reported that food-borne transmission of HEV may occur through ingestion of raw or undercooked meat including liver and intestine from infected swine, deer or boar [Matsuda et al., 2003; Tei et al., 2003; Yazaki et al., 2003]. Imported hepatitis E and transfusion-transmitted HEV infection have also been documented [Koizumi et al., 2004; Matsubayashi et al., 2004; Mitsui et al., 2004]. Furthermore, a high prevalence of IgG class antibodies to HEV (anti-HEV IgG) among healthy individuals, most likely due to past subclinical HEV infection, has been reported in some regions in Japan [Li et al., 2000; Tanaka et al., 2001, 2005; Mitsui et al., 2004, 2005], and HEV-viremic subjects have been identified among symptom-free blood donors with an elevated alanine aminotransferase (ALT) level [Fukuda et al., 2004]. However, it remains unknown whether and, if so, how frequently recent subclinical HEV infection is occurring in Japan. Therefore, in an attempt to estimate the prevalence of recent subclinical HEV infection in Japan stratified by ALT level, anti-HEV antibodies and HEV RNA were assayed in serum samples obtained from Japanese voluntary blood donors with an elevated ALT level of ≥61 IU/l who are likely to have ongoing HEV infection.

MATERIALS AND METHODS

Serum Samples

Approximately 2.1% of voluntary blood donors had an elevated ALT level of 61 IU/l or greater at the Japanese Red Cross Saitama Blood Center, Japan, between April 2003 and March 2006. During this period, serum samples were collected from a total of 6700 voluntary blood donors (age, 35.7 ± 10.6 [mean ± standard deviation, SD] years; 6051 men and 649 women) with an elevated ALT level of 61–476 (range; 88.9 ± 34.6, mean ± SD) IU/l. The Blood Center is located in Saitama Prefecture, a prefecture in the central part of mainland Honshu of Japan.

All 6700 donors were negative for hepatitis B surface antigen and antibodies to hepatitis C virus (HCV), human immunodeficiency virus (HIV) types 1 and 2, and human T-lymphotropic virus type 1, as well as hepatitis B virus DNA, HCV RNA and HIV type 1 RNA by the nucleic acid amplification test using Roche's Multiplex reagent [Mine et al., 2003]. Serum samples obtained from repeat donors during the study period were excluded: that is, each sample was obtained from a unique individual.

Detection of Antibodies to HEV

To detect anti-HEV IgG, anti-HEV IgM and anti-HEV IgA, enzyme-linked immunosorbent assay (ELISA) was performed using purified recombinant ORF2 protein of HEV genotype 4 that had been expressed in the pupae of silkworm [Mizuo et al., 2002], as described previously [Takahashi et al., 2005]. The optical density (OD) of each sample was read at 450 nm. The cut-off value used for the anti-HEV IgG, anti-HEV IgM, and anti-HEV IgA assays was 0.175, 0.440, and 0.642, respectively [Takahashi et al., 2005]. Samples with OD values for anti-HEV IgG, IgM, or IgA equal to or greater than the respective cut-off value were considered to be positive for anti-HEV IgG, IgM, or IgA, respectively. The specificity of the anti-HEV assays was verified by absorption with the same recombinant ORF2 protein that was used as the antigen probe. Briefly, if the OD value of the tested sample was less than 30% of the original value after absorption with the recombinant ORF2 protein, the sample was considered to be positive for anti-HEV.

Detection of HEV RNA

In serum samples with anti-HEV IgM and/or anti-HEV IgA, reverse transcription (RT)-polymerase chain reaction (PCR) was performed for detection of HEV RNA using nested primers targeting the ORF2 region as described previously [Mizuo et al., 2002]. The size of the amplification product of the first-round PCR was 506 base pairs (bp), and that of the second-round PCR was 457 bp. The nested RT-PCR assay that we used is capable of amplifying all four known genotypes of HEV strains reported thus far [Mizuo et al., 2002; Takahashi et al., 2003b; Yazaki et al., 2003]. The specificity of the RT-PCR assay was verified by sequence analysis as described below. The sensitivity of the RT-PCR assay was assessed as described previously [Mizuo et al., 2002]. For serum samples that were negative for HEV RNA when 100 μl of serum samples was used, total RNA was extracted from 500 μl of serum, reverse transcribed, and then subjected to the nested PCR as described above. To extract RNA from 500 μl of serum, test serum diluted two-fold in saline was centrifuged at 287,582 × *g* at 4°C for 2 hr in a TLA-100.2 rotor (Beckman Coulter K.K., Tokyo, Japan), and the resulting pellet was suspended in 100 μl of saline and subjected to the RT-PCR assay. To confirm the reproducibility, this assay was performed in duplicate.

As for serum samples without anti-HEV IgM and anti-HEV IgA, 10 μl each from 10 serum samples were pooled,

and each pool was tested for HEV RNA by the above-mentioned RT-PCR assay. If a pool was positive for HEV RNA, the 10 serum samples of that pool were individually tested for the presence of HEV RNA. This RT-PCR assay was performed using both 10 and 100 μl of each serum sample, and reproducibility was confirmed.

Sequence Analysis of PCR Products

The amplification products were sequenced directly on both strands using the BigDye Terminator Cycle Sequencing Ready Reaction Kit on an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequence analysis was performed using Genetyx-Mac Version 12.2.7 (Genetyx Corp., Tokyo, Japan) and ODEN Version 1.1.1 from the DNA Data Bank of Japan (DDBJ: National Institute of Genetics, Mishima, Japan) [Ina, 1994]. Sequence alignments were generated by CLUSTAL W (version 1.8) [Thompson et al., 1994]. A phylogenetic tree was constructed by the neighbor-joining method [Saitou and Nei, 1987] based on the partial nucleotide sequence of the ORF2 region (412 nucleotides [nt]). Bootstrap values were determined on 1000 resamplings of the data sets [Felsenstein, 1985].

Statistical Analysis

Statistical analyses were performed using the χ^2-test for comparison of proportions between two groups. Differences were considered to be statistically significant at $P < 0.05$.

RESULTS

Age- and Sex-Specific Prevalence of Anti-HEV Antibodies

A total of 6700 serum samples obtained from apparently healthy blood donors with an elevated ALT level were tested for the presence of anti-HEV IgG. Anti-HEV IgG was detected in 7.1% (479/6700) of the tested population including 7.0% of the 6051 male donors and 8.6% of the 649 female donors, the difference not being significant (Table I). The prevalence of anti-HEV IgG generally increased with age among both the male and female donors, and was significantly higher among donors aged ≥30 years than among those aged <30 years in total (8.8% vs. 2.9%, $P < 0.0001$), in the males (8.6% vs. 2.9%, $P < 0.0001$) and in the females (10.7% vs. 2.5%, $P = 0.0013$). The 479 serum samples with anti-HEV IgG were tested for anti-HEV IgM and anti-HEV IgA. Among them, anti-HEV IgM was detected in eight samples (1.7%) and anti-HEV IgA in seven samples (1.5%). In total, nine samples were positive for anti-HEV IgM and/or anti-HEV IgA (Table II).

Detection of HEV RNA Among All 6700 Blood Donors

Among the nine serum samples with anti-HEV IgM and/or anti-HEV IgA, five samples tested positive for HEV RNA when RT-PCR was performed with a sample volume of both 10 and 100 μl, and one sample was positive for HEV RNA with a sample volume of 500 μl (Group A in Table II). Among 661 10-sample pools and nine 9-sample pools, three 10-sample pools were positive for HEV RNA. The 30 serum samples of the 3 pools that had been positive for HEV RNA were tested individually for the presence of HEV RNA, and 3 samples (nos. 5503, 8177 and 7369 in Groups B and C in Table II) were found to be positive for HEV RNA in two distinct volumes of 10 and 100 μl. Consequently, 9 (0.1%) of the 6700 samples were found to be viremic for HEV in the present study. When stratified by the presence of anti-HEV antibodies, HEV RNA was detectable in 6 (66.7%) of the 9 donors with anti-HEV IgM and/or anti-HEV IgA, 2 (0.4%) of the 470 donors with anti-HEV IgG but without anti-HEV IgM or anti-HEV IgA, and 1 (0.02%) of the 6221 donors without any serological markers of HEV infection.

Prevalence of Anti-HEV and HEV RNA, Stratified by ALT Level

In the present study, 479 donors with anti-HEV IgG were found, including 371 (7.2%) with an ALT level of 61–100 IU/l, 96 (6.6%) with an ALT level of 101–200 IU/l, and 12 (11.0%) with an ALT level of ≥201 IU/l (Table III). The prevalence of anti-HEV IgG was higher among donors with an ALT level of ≥201 IU/l than among those with an ALT level of 61–200 IU/l, although the difference was not statistically significant (11.0% vs. 7.1%, $P = 0.1148$). As for the prevalence of HEV RNA, there was a significant difference between donors with an ALT level of ≥201 IU/l and those with an ALT level of 61–200 IU/l in total (2.8% vs. 0.1%, $P < 0.0001$), in males (2.1% vs. 0.1%, $P < 0.0001$) and in females (8.3% vs. 0%, $P < 0.0001$).

Genetic Analysis of HEV Isolates Recovered from Nine Viremic Donors

The nine HEV isolates recovered from the transiently viremic donors were named HE-JSB1217, HE-JSB1564, HE-JSB1582, HE-JSB4175, HE-JSB5503, HE-JSB6151, HE-JSB7017, HE-JSB7369, and HE-JSB8177, respectively, with the prefix of HE-JSB followed by the ID no. of each sample. The 412-nt sequence of ORF2 of these HEV isolates were determined and compared with each other and with that of known HEV isolates of genotypes 1–4. These nine HEV isolates were markedly variable, sharing nucleotide identities ranging from 85.6% to 98.5%. However, they were all close to the prototype Japanese isolate of genotype 3 (JRA1 [accession no. AP003430]) with nucleotide identities of 87.3–93.9%, and were only 78.1–80.7%, 75.2–76.6%, and 78.2–80.5% similar to the B1 isolate (M73218) of genotype 1, MEX-14 isolate (M74506) of genotype 2, and T1 isolate (AJ272108) of genotype 4, respectively. When the nine HEV isolates obtained in the present study were compared with 412 other reported genotype 3 isolates whose common 299-to-412-nt ORF2 sequence is available as of December 2006, each of them was closest to a human or swine HEV

TABLE I. Age- and Sex-Dependent Prevalence of Anti-HEV Antibodies

	Total			Male			Female		
		Anti-HEV			Anti-HEV			Anti-HEV	
Age (years)	N	IgG-class	IgM- and/or IgA-class	N	IgG-class	IgM- and/or IgA-class	N	IgG-class	IgM- and/or IgA-class
16–19	439	13 (3.0%)	0	397	11 (2.8%)	0	42	2 (4.8%)	0
20–29	1414	40 (2.8%)	0	1294	38 (2.9%)	0	120	2 (1.7%)	0
30–39	2736	183 (6.7%)	4 (0.1%)	2541	164 (6.5%)	3 (0.1%)	195	19 (9.7%)	1 (0.5%)
40–49	1319	126 (9.6%)	3 (0.2%)	1202	114 (9.5%)	3 (0.2%)	117	12 (10.3%)	0
50–59	651	93 (14.3%)	1 (0.2%)	520	77 (14.8%)	1 (0.2%)	131	16 (12.2%)	0
60–70	141	24 (17.0%)	1 (0.7%)	97	19 (19.6%)	1 (1.0%)	44	5 (11.4%)	0
Total	6700	479 (7.1%)	9 (0.1%)	6051	423 (7.0%)	8 (0.1%)	649	56 (8.6%)	1 (0.2%)

TABLE II. Detection of HEV RNA Among Three Categories of Blood Donors With Elevated ALT Level

					Anti-HEV (OD_{450} value)			HEV RNA[a]		
ID no.	Age (years)/ sex	ALT (IU/l)	AST (IU/l)	γ-GTP (IU/l)	IgG-class	IgM-class	IgA-class	10 µl	100 µl	500 µl
Group A ($n=9$) with anti-HEV IgG with anti-HEV IgM and/or anti-HEV IgA										
1217	34/M	94	37	149	2.583 (+)	1.818 (+)	1.676 (+)	+[b]	+	NT[c]
1564	48/M	61	32	86	2.343 (+)	1.709 (+)	2.648 (+)	+	+	NT
1582	51/M	101	52	89	2.404 (+)	0.917 (+)	1.310 (+)	+	+	NT
4175	68/M	261	145	154	1.709 (+)	2.566 (+)	1.130 (+)	+	+	NT
6151	49/M	128	41	607	1.364 (+)	2.237 (+)	2.442 (+)	+	+	NT
7017	36/F	224	95	362	1.401 (+)	1.313 (+)	0.348 (−)	−	−	+
1304	38/M	181	67	104	>3.000 (+)	>3.000 (+)	0.825 (+)	−	−	−
3243	35/M	170	78	334	0.404 (+)	0.470 (+)	0.017 (−)	−	−	−
7667	47/M	66	37	45	0.350 (+)	0.044 (−)	0.868 (+)	−	−	−
Group B ($n=470$) with anti-HEV IgG but without anti-HEV IgM nor anti-HEV IgA										
5503	34/M	77	55	20	0.394 (+)	0.091 (−)	0.138 (−)	+	+	NT
8177	41/M	82	53	197	0.193 (+)	0.042 (−)	0.061 (−)	+	+	NT
Group C ($n=6221$) without anti-HEV IgG										
7369	48/M	276	210	219	0.006 (−)	0.016 (−)	0.011 (−)	+	+	NT

[a]HEV RNA was assayed using the indicated volume of serum sample.
[b]+, positive for HEV RNA; −, negative for HEV RNA.
[c]NT, not tested.

TABLE III. Prevalence of Anti-HEV IgG and HEV RNA Among Voluntary Blood Donors With Elevated ALT Level, Stratified by ALT Level

	Total			Male			Female		
ALT (IU/l)	N	Anti-HEV IgG	HEV RNA	N	Anti-HEV IgG	HEV RNA	N	Anti-HEV IgG	HEV RNA
61–100	5131	371 (7.2%)	4 (0.1%)	4635	331 (7.1%)	4 (0.1%)	496	40 (8.1%)	0
101–200	1460	96 (6.6%)	2 (0.1%)	1319	83 (6.3%)	2 (0.2%)	141	13 (9.2%)	0
201–476	109	12 (11.0%)	3 (2.8%)	97	9 (9.3%)	2 (2.1%)	12	3 (25.0%)	1 (8.3%)
Total	6700	479 (7.1%)	9 (0.1%)	6051	423 (7.0%)	8 (0.1%)	649	56 (8.6%)	1 (0.2%)

isolate of Japan origin. That is, HE-JSB1564, HE-JSB5503, and HE-JSB8177 had the highest identity of 99.0%, 98.5% and 98.3%, respectively, with HE-JBD2 (AB154829). The HE-JSB4175 isolate was closest to HE-JA9 (AB082565, 97.8%), HE-JSB1217 and HE-JSB1582 to HE-JHD1988 (AB175485, 94.9% and 93.7%, respectively), HE-JSB7369 to HE-JA21 (AB115542, 94.2%), HE-JSB6151 to G3-4531-Swine (DQ079632, 94.4%), and HE-JSB7017 to HE-JHD1980 (AB175484, 93.4%).

The phylogenetic tree constructed based on the common 412-nt sequence within the ORF2 sequence confirmed that the nine HEV isolates obtained in the present study belonged to genotype 3, and that they segregated into the clusters consisting of Japanese HEV strains of the same genotype that had been recovered from humans, swine and wild boars, supporting the indigenous nature of these nine blood donor isolates (Fig. 1).

DISCUSSION

Recent studies have documented that sporadic acute hepatitis E does occur among individuals in industrialized countries with no history of travel to areas endemic for HEV [Kwo et al., 1997; Harrison, 1999; Mansuy et al., 2004; Ijaz et al., 2005; Waar et al., 2005; Amon et al., 2006; Preiss et al., 2006; Sadler et al., 2006]. In Japan, hepatitis E is rare compared with hepatitis A, but is occurring more frequently than previously thought [Mizuo et al., 2002; Okamoto et al., 2003]. As for the geographical distribution of hepatitis E in Japan, it was reported that there was wide variation with a higher prevalence in the northern part of Japan (Hokkaido Island and northern part of mainland Honshu) [Mizuo et al., 2002; Abe et al., 2006]. The Japanese Red Cross Saitama Blood Center is located in Saitama Prefecture, which is north of and adjacent to Metropolitan Tokyo. Only six cases of locally acquired sporadic acute hepatitis E have thus far been reported in this prefecture, in contrast with more than 100 cases in Hokkaido and 23 cases in Tokyo [Abe et al., 2006].

In the present study, 7.0% (468/6700) of the study population had anti-HEV IgG in the absence of IgM/IgA class anti-HEV and HEV RNA, which is much higher than expected. This finding suggests the presence of frequent past HEV infection among individuals living in the central part of Japan, most of which seem to be subclinical. HEV RNA was assayed in serum samples obtained from all 6700 donors, and the prevalence of ongoing subclinical HEV infection in three distinct groups of the study population according to the presence of class-specific HEV antibodies, was investigated. Among the nine donors with IgM and/or IgA class anti-HEV, six donors (66.7%) were found to be viremic for HEV. Furthermore, among the 6691 donors without anti-HEV IgM and anti-HEV IgA, three HEV-viremic donors with no signs or symptoms of hepatitis were found. In an attempt to detect HEV RNA in a large sample size, we first screened for present HEV infection by testing 10-sample minipools (each pool contained

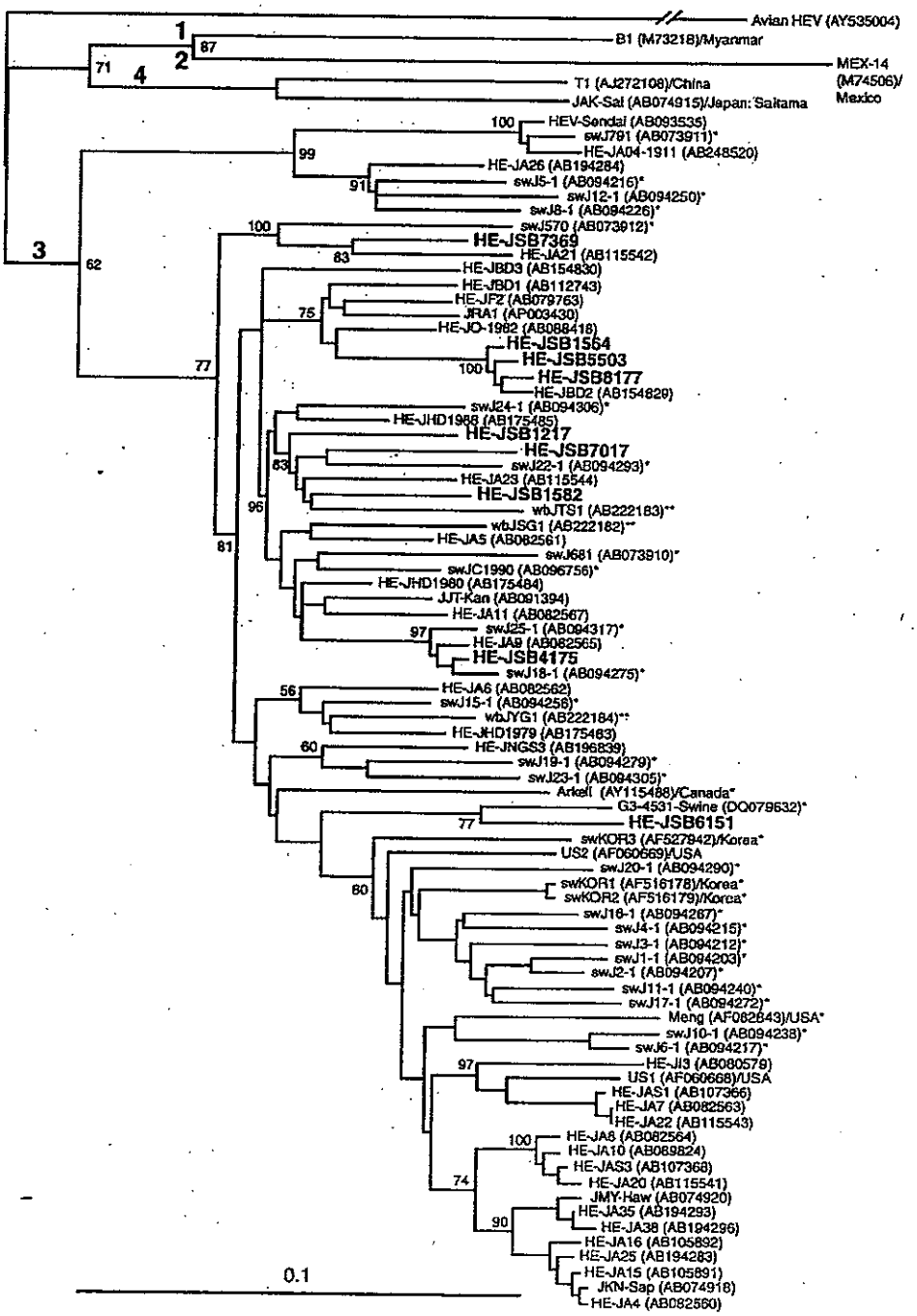

Fig. 1. Phylogenetic tree constructed by the neighbor-joining method based on the partial nucleotide sequence of the ORF2 region (412 nt) of 84 HEV isolates, using a chicken HEV (AY535004) as an outgroup. In addition to the HE-JSB1217, HE-JSB1564, HE-JSB1582, HE-JSB4175, HE-JSB5503, HE-JSB6151, HE-JSB7017, HE-JSB7369, and HE-JSB8177 isolates found in the present study which are indicated in bold type, 75 reported HEV isolates of genotypes 1–4 whose common 412-nt sequence is known are included for comparison and their accession nos. are shown in parentheses. Swine and wild boar HEV isolates are indicated with asterisks (* and **, respectively). Bootstrap values are indicated for the major nodes as a percentage obtained from 1000 resamplings of the data.

10 μl of serum from each of 10 subjects); then, for pools that were positive for HEV RNA, the individual serum samples were tested for the presence of HEV RNA. Despite the limited amount of serum from each sample that was tested, current subclinical HEV infection was recognized molecularly in three donors in this study. Two viremic donors (0.4%) were found among the 470 donors with anti-HEV IgG but without anti-HEV IgM nor anti-HEV IgA. Since serial serum samples were not available from these two viremic donors during the HEV infection, it is unclear whether they could not elicit acute antibodies of anti-HEV IgM and anti-HEV IgA.

However, the observation that HEV RNA is detectable in serum despite the absence of anti-HEV IgM and anti-HEV IgA is in agreement with our previous observations that two hemodialysis patients and one hospital employee who contracted subclinical HEV infection in 1979, 1980, or 2003, respectively, exhibited only anti-HEV IgG, although transient HEV viremia was observed [Mitsui et al., 2004, 2005]. Patients with HEV infection without an acute antibody response have also been reported [Caudill et al., 1994; Clayson et al., 1995].

Of particular note, one donor (0.02%) was found to have HEV RNA among the 6221 donors without serological markers of HEV infection in the present study. The precise reason why the viremic donor did not show an antibody response against HEV despite significant elevations of ALT (276 IU/l) and AST (210 IU/l) levels is unknown. However, 4 months before the blood sampling, he had elevated ALT (81 IU/l) and AST (165 IU/l) levels in the absence of HEV RNA in serum, suggesting that he contracted subclinical HEV infection on the background of an unexplained chronic liver disease. It was reported that neither anti-HEV IgG nor anti-HEV IgM was detectable in four symptom-free persons with evidence of HEV viremia who came into contact with patients with acute hepatitis E during an outbreak of hepatitis E [Nicand et al., 2001]. Aggarwal et al. [2001] conducted experimental studies on subclinical HEV infection in cynomolgus macaques, and reported that subclinical HEV infection in some animals was associated with failure of the development of an immune response, compared with animals with clinical HEV infection.

In Japan, approximately 200 patients with clinical HEV infection and 80 patients with subclinical HEV infection who contracted the infection between 2001 and 2005 have been reported [Okamoto et al., 2003; Fukuda et al., 2004; Abe et al., 2006; Inoue et al., 2006]. However, the exact ratio of the number of cases of clinical HEV infection to that of subclinical HEV infection remains unknown. At the Japanese Red Cross Saitama Blood Center, 2.1% of voluntary blood donors had an elevated ALT level of 61 IU/l or greater during the period from April 2003 to March 2006. The population size of individuals ≥20 years of age in Saitama Prefecture was reported to be 5.68 million on January 1, 2005 (http://www.pref.saitama.lg.jp/). In the present study, 9 (0.13%) of 6700 individuals with an elevated ALT level of ≥61 IU/l had HEV viremia. Assuming that HEV viremia is detectable by RT-PCR for 1 month during acute HEV infection [Takahashi et al., 2003a, 2005], the annual number of cases of subclinical HEV infection in Saitama Prefecture is estimated to be approximately 2000. Although we cannot rule out the possibility that the number of cases with clinical HEV infection is underestimated, only one or two patients with hepatitis E have been reported per year in this prefecture. Therefore, it is assumed that less than 0.1% of HEV-infected cases exhibit clinical manifestation of the infection.

As the nine viremic donors identified in the present study had an elevated ALT level, the blood from the nine donors was not used for transfusion, suggesting that ALT testing may help prevent transfusion-transmitted HEV infection. As one of the nine infected donors had only a slightly elevated ALT level of 61 IU/l, it seems likely that even donors with a normal ALT level (≤60 IU/l) may have detectable HEV RNA. However, the prevalence of HEV RNA decreased with ALT level, and was significantly lower among the 5131 donors with ALT level of 61–100 IU/l than among the 109 donors with ALT of ≥201 IU/l (0.078% vs. 2.8%, $P < 0.0001$). It is reasonable to speculate that the prevalence of ongoing HEV infection among donors with a normal ALT level may be less than 0.078% in Saitama Prefecture. The proportion of such donors may be significantly small or negligible, according to the geographic region. Reflecting the high prevalence of clinical HEV infection, at least three cases of transfusion-transmitted hepatitis E have been reported in Hokkaido and one case in Tokyo [Matsubayashi et al., 2004; Abe et al., 2006], but none in Saitama Prefecture up to the present. As donors with normal ALT level were not tested for HEV viremia in the present study, we cannot conclude that the serum ALT level can be used to exclude blood donors with ongoing HEV infection. Based on the current study, however, we would consider that ALT testing is at least useful in part for exclusion of donors with HEV viremia with the aim of preventing transfusion-associated hepatitis E, although alcohol consumption and obesity should be taken into consideration as the major contributing factors to an elevated ALT level in blood donors.

Multiple HEV strains of genotype 3 or 4 have been isolated from Japanese patients with sporadic acute or fulminant hepatitis E as well as from farm pigs, wild boars, a wild deer and a mongoose in Japan [Mizuo et al., 2002; Takahashi et al., 2003a,b, 2004; Inoue et al., 2006; Nakamura et al., 2006]. Reflecting the polyphyletic nature of human and animal HEV isolates of Japan origin, the HEV isolates recovered from nine viremic donors in the present study, differed by 1.5–14.4% from each other, although they belonged to the same genotype (genotype 3) with the highest nucleotide sequence identity of 87.3–93.9% with the JRA1 isolate that is believed to be indigenous to Japan [Takahashi et al., 2001]. A human HEV strain of genotype 4 (JAK-Sai [AB074915]) has been isolated [Takahashi et al., 2002] in the same prefecture as that of the nine viremic donors, and it shares only 78.6–81.3% identities with the nine HEV isolates obtained from the viremic donors in the present study. These results further support the marked heterogeneity of the HEV genome and its wide distribution in Japan, even within a certain prefecture in this country.

In conclusion, nine blood donors with HEV viremia were identified among 6700 voluntary blood donors with an elevated ALT level at a blood center located in the central part of mainland Honshu of Japan where hepatitis E is low-endemic. This study indicates that approximately 0.1% of individuals with an elevated ALT

level and 3% of individuals with an ALT level of ≥201 IU/l have ongoing subclinical infection of various HEV strains, suggesting the frequent occurrence of subclinical HEV infection, although clinical HEV infection is rarely reported. A large study of individuals who do not have an elevated ALT level is needed to assess the exact frequency of subclinical HEV infection, taking into consideration the geographic region in Japan.

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識別番号・報告回数			報告日	第一報入手日 2007 年 5 月 7 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	人ハプトグロビン	研究報告の公表状況	Medical Molecular Morphology 2007; 40(1): 23-28	公表国 日本
販売名 (企業名)	ハプトグロビン注ーヨシトミ(ベネシス)			

研究報告の概要

GBV-C 及び HGV は、非 B 非 C 型肝炎の原因として関与する新たなウイルスとして紹介されたが、これらウイルス粒子の形態は依然不明であるとともに、非 A-E 肝炎の殆どの症例は、それらの感染と関係がない。我々は、ALT が高く、HCV 抗体と B 型肝炎表面抗原が陰性であるドナーからの血漿サンプル中のウイルス様粒子を視覚的に捉えようと試み、このウイルス様粒子と非経口的に感染する GBV-C/HGV の遺伝子との関係を調査した。
HBV 及び HCV どちらにも感染していない高 ALT レベルの血漿 13 検体及び正常 ALT レベルの血漿 10 検体の計 23 検体を 40-60%ショ糖密度勾配遠心分離法によって分離し、ウイルス様粒子(VLPs)を電子顕微鏡で観察した。血漿中の GBV-C/HGV RNA を検査した。ウイルス様粒子は、高 ALT レベルの血漿 13 検体のうち 12 検体 (92.3%) 及び正常対照血漿 10 検体のうち 1 検体 (10%) の 1.15-1.16g/mL の密度のフラクションから見つかった。VLPs の超微細構造形態は、大きさ、外観とも多形性であった (大部分の VLPs は 35-45nm の内核と表面に長さ 9-12nm のスパイク様突起を有する 50-80nm の球状粒子)。直径 50-70nm、長さ 110-160nm の棒状の VLPs も同じ検体で観察された。循環血液中の VLPs の検出率は、有意に高 ALT レベルと関係 ($P<0.001$) 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。HBV 及び HCV ともに陰性の血漿中に高 ALT との有意な関係が認められた球状の 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。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-I 抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
非 B 非 C 型肝炎との関連が示唆されるウイルス様粒子を発見したとの報告である。 非 B 非 C 型肝炎との関連が示唆されるウイルス様粒子は、GBV-C/HGV と関連していないこと以外の性状等に関する情報は不足しているため、現時点において安全性評価は困難であり、今後も情報収集に努める。	ウイルス様粒子に関する追加情報の入手に努める。

(17)

Med Mol Morphol (2007) 40:23–28
DOI 10.1007/s00795-006-0342-7

ORIGINAL PAPER

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Unidentified virus-like particles are detected in plasmas with elevated ALT levels: are they significant of etiological agent(s) of non-B, non-C hepatitis?

Received: May 9, 2006 / Accepted: June 22, 2006

Abstract GB virus C (GBV-C) and hepatitis G virus (HGV) have been proposed as new viruses etiologically implicated in non-B, non-C hepatitis, but the morphology of these particular virus particles is still unknown, and most cases of non-A to E hepatitis do not relate to their infections. We tried to visualize virus-like particles (VLPs) in plasma samples from hepatitis B surface antigen- and antibody to hepatitis C virus (HCV)-negative blood donors with elevated alanine aminotransferase (ALT), and examined the association of the virus-like particles and the genomes of parenterally transmissible GBV-C/HGV. Twenty-three plasma samples, 13 with elevated ALT levels and 10 with normal ALT values, from blood donors without infections of hepatitis B virus (HBV) and HCV, were subjected to a 20%–60% sucrose density gradient centrifugation, and virus-like particles were observed by electron microscopy. GBV-C/HGV RNAs in the plasmas were tested. Virus-like particles were found in the fractions with densities of 1.15–1.16 g/ml from 12 of 13 (92.3%) plasmas with elevated ALT levels and 1 of 10 (10%) normal controls. The ultrastructural morphology of visualized VLPs was pleomorphic in size and appearance; the majority of the VLPs were 50- to 80-nm spherical particles with a 35- to 45-nm inner core and 9- to 12-nm-long surface spikelike projections. Rodlike VLPs 50–70 nm in diameter with a length of 110–160 nm were also observed in the same samples. The incidence of detection of the circulating VLPs was significantly ($P < 0.001$) related to elevated ALT levels, but GBV-C/HGV RNAs were detected in none of the plasmas containing the virus-like particles. Spherical VLPs are detected in HBV- and HCV-negative plasmas significantly correlated with the elevation of ALT, suggesting that they are implicated in non-B, non-C hepatitis.

Key words Non-B, non-C hepatitis · Virus-like particle · Hepatitis C virus · GB virus C · Electron microscopy

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Introduction

The genomes of hepatitis C virus (HCV), GB virus C (GBV-C), and hepatitis G virus (HGV) have been successfully cloned without isolation of the virus particles.[1–3] The causative role of HCV in bloodborne acute and chronic hepatitis has been well established. Although GBV-C/HGV can be transmitted parenterally, most of their infections are not associated with acute and chronic non-B, non-C hepatitis, and there is some doubt whether GBV-C/HGV replicates in the liver and causes hepatitis.[4,5] During immunogold electron microscopy[6–9] of HBV and HCV particles, we have noticed that some plasma samples from blood donors with elevated alanine aminotransferase (ALT) levels contained virus-like particles (VLPs) that did not react positively with antibodies specific to the HCV envelope protein. The VLPs, thus immunologically distinguished from HCV virion, were also different in morphology from the latter, and their etiological implications were not clear. In this article, we have tried to visualize VLPs in hepatitis B surface antigen (HBsAg)- and anti-HCV-negative plasmas and evaluated whether the circulating VLPs were detected with or without a relationship to elevated plasma ALT levels or to GBV-C/HGV RNAs.

Materials and methods

Twenty-three blood donor plasma samples were the subjects in this study. Clinical characteristics of the blood donors are summarized in Table 1. These samples were negative for HBsAg (AUSRIA II-125; Dainabot, Tokyo,

Table 1. Summarized data of detection of virus-like particles and nucleic acids of known hepatitis viruses in plasma samples negative for HBsAg and anti-HCV

Plasma no.	ALT (IU/l)	Virus-like particles[a]		HBV DNA[b]	HCV RNA[c]	GBV-C RNA[d]	HGV RNA[e]
		Spherical	Rod-like				
1	91	–	–	–	–	–	–
2	81	2+	–	–	–	ND	ND
3	87	2+	–	–	–	–	–
4	70	3+	2+	–	–	–	–
5	82	3+	2+	–	–	–	–
6	103	3+	1+	–	–	–	–
7	121	3+	3+	–	–	–	–
8	67	2+	1+	–	–	–	–
9	117	2+	–	–	–	–	–
10	72	2+	1+	–	–	–	–
11	103	2+	–	–	–	–	–
12	78	3+	1+	–	–	ND	ND
13	77	1+	–	–	–	–	–
14	4	1+	–	–	–	–	–
15	28	–	–	–	–	–	–
16	6	–	–	–	–	–	–
17	10	–	–	–	–	–	–
18	7	–	–	–	–	–	–
19	11	–	–	–	–	–	–
20	4	–	–	–	–	–	–
21	6	–	–	–	–	–	–
22	6	–	–	–	–	–	–
23	8	–	–	–	–	–	–

ALT, alanine aminotransferase (normal < 35 IU/L); ND; not dated
[a] Circulating virus-like particles (VLPs) in a 5-square section of a 300-mesh grid was photographed under electron microscopy. The mean numbers of VLPs in one square was indicated as – (negative), 1+ ($0 < + < 10$), 2+ ($10 \leqq 2+ < 100$), and 3+ ($100 \leqq 3+$)
[b] HBV DNA was tested using a Quantiplex HBV-DNA assay kit
[c] HCV RNA was tested using an Amplicor HCV kit
[d] GBV-C and [e] HGV RNAs were assayed as described in the text

Japan), antibody to HBV core antigen (CORAB; Dainabot), HBV DNA (Quantiplex HBV-DNA Assay; Chiron, Emeryville, CA, USA), anti-HCV (Ortho HCV Ab IRMA Test III; Ortho-Clinical Diagnostics, Tokyo, Japan), HCV RNA (Amplicor HCV; Roche Molecular Systems, Branchburg, NJ, USA), and antibodies to human T-cell leukemia virus type I (Determiner HTLV-I Antibody; Kyowa Medex, Tokyo, Japan) and human immunodeficiency virus (GENELAVIA MIXT; Sanofi Diagnostics Pasteur, Marnes la Coquette, France). Thirteen samples were elevated in alanine aminotransferase (ALT) levels (≥35 IU/l), and 10 samples were within a normal level in ALT (<35 IU/l). Total cholesterol and triglyceride levels were normal in all plasma samples.

One hundred milliliters of each plasma sample was diluted with two volumes of TEN [100 mM Tris-HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA), 100 mM NaCl] and centrifuged at 75 000 *g* for 6 h at 4°C. A suspension of the pellet in TEN was centrifuged again at 150 000 *g* for 2.5 h at 4°C. An approximately 1000-fold-concentrated suspension of the sample in TEN was layered on a 20%–60% (W/W) linear sucrose density gradient in TE (100 mM Tris-HCl, pH 8.0, 1 mM EDTA) and centrifuged at 100 000 *g* for 16 h at 4°C, which was followed by fractionation from the bottom of the tube. After measurement of the sucrose concentration of every fraction, each sucrose fraction was diluted in phosphate-buffered saline (PBS, pH 7.4) and then centrifuged at 150 000 g for 2.5 h at 4°C. The resulting pellet was suspended in 100 ml PBS, equivalent to 1:1000 of the original plasma volume, for observing VLPs, and stored at –80°C until use. Two to three microliters of the concentrated specimen was mounted on a Formvar-coated and carbon-vaporized copper grid and examined under a Hitachi H-800 electron microscope operated at 100 kV after staining with 2% phosphotungstic acid (pH 6.5). To observe the nucleic acid-containing virus core structure, the specimen was subjected to repeated freezing and thawing several times, and then stained with 0.2% uranyl acetate (pH 4.4).

The GBV-C genome was assayed by reverse transcription-nested polymerase chain reaction (PCR) using primers derived from the NS3/helicase region of GBV-C genome reported by Simons et al.,[2] and this assay was performed as described elsewhere.[10] The genome of HGV, provisionally designated by Linnen et al.,[3] was assayed by using the kit of Boehringer Mannheim (Mannheim, Germany), which contained one primer pair for the NS5A region and a second primer pair for the 5′-untranslated region as well as the corresponding capture probes, according to the manufacturer's instructions.

Fisher's exact probability test was used to assess the significance of differences between the sample groups with or without elevated ALT levels.

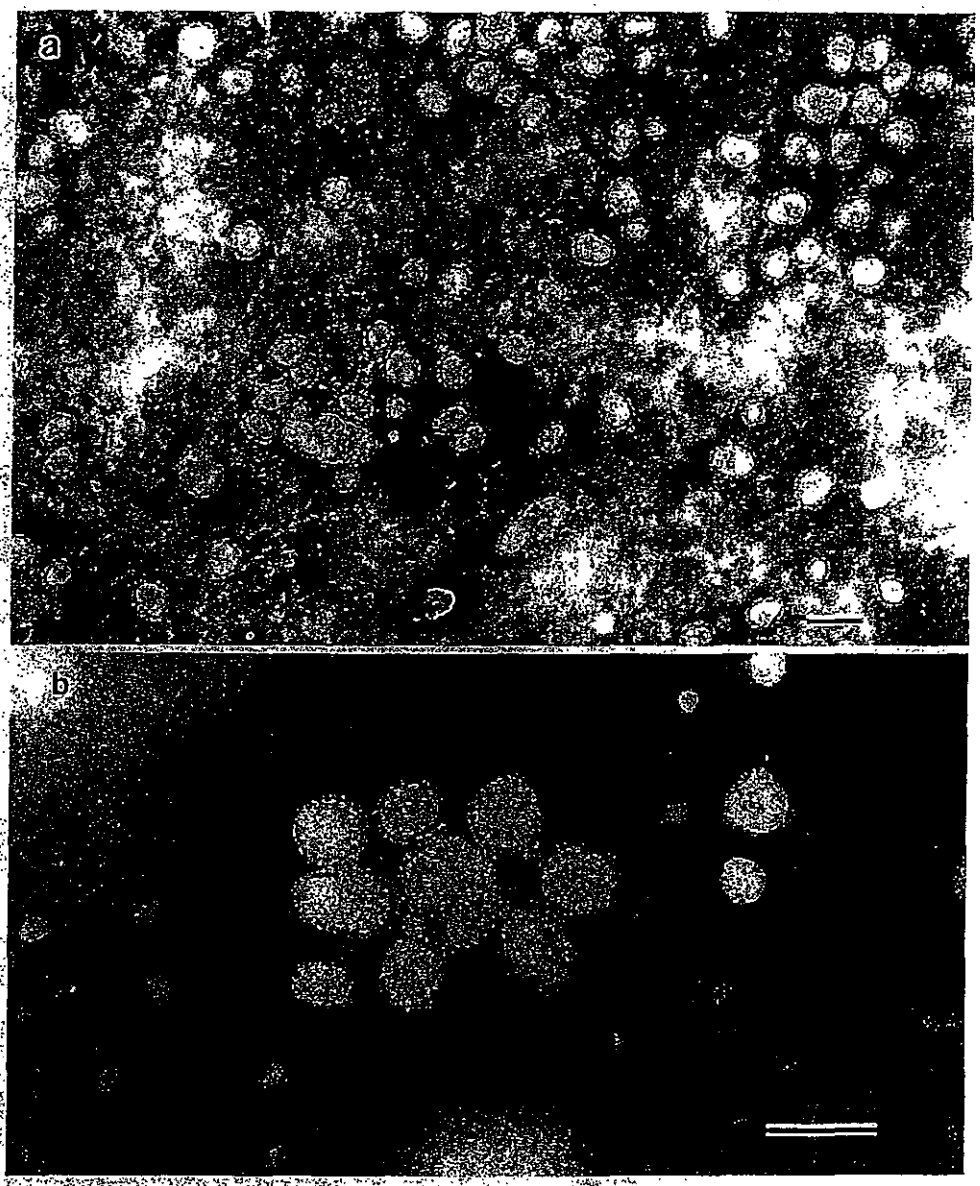

Fig. 1. Electron micrograph of negatively stained 50- to 80-nm spherical virus-like particles (VLPs) in hepatitis B surface antigen (HBsAg)- and anti-hepatitis C virus (HCV)-negative plasma with an elevated alanine aminotransferase (ALT) level. VLPs from sample no. 6 (a) and sample no. 3 (b) are shown. *Bar* 100 nm

Results

Spherical VLPs were detected in 12 of 13 (92.3%) plasma samples with elevated ALT levels and 1 of 10 (10%) plasma samples with normal ALT values. The incidence of detection of the virus-like particles was significantly ($P < 0.001$) related to the elevation of plasma ALT levels (see Table 1).

Most the visualized VLPs were spherical particles 50–80 nm in diameter with 9- to 12-nm-long surface spikelike projections (Fig. 1a,b). Some VLPs have a large diameter, more than 100 nm (Fig. 1a). In some specimens were detected more than a moderate number of VLPs, and rodlike VLPs 50–60 nm in diameter with a length of 110–160 nm were also detected that had surface projections similar to those of the 50- to 80-nm spherical particles (Fig. 2). The diameter of the rodlike VLPs was 50–70 nm and their length was 110–160 nm. In addition, a 35- to 45-nm corelike structure containing the electron-dense material within a spherical VLP (Fig. 3a,b) and two inner cores of a rodlike VLP (Fig. 3c) were visualized by positive staining with uranyl acetate. These VLPs were found to be constantly banded in a sucrose density gradient at around 1.15–1.16 g/ml (range, 1.12–1.18 g/ml). GBV-C/HGV RNAs were not detected in any of the plasma samples (see Table 1).

Discussion

We found VLPs in ALT-elevated plasma samples from blood donors without infections of HBV and HCV that were consistently banded at around 1.15–1.16 g/ml by

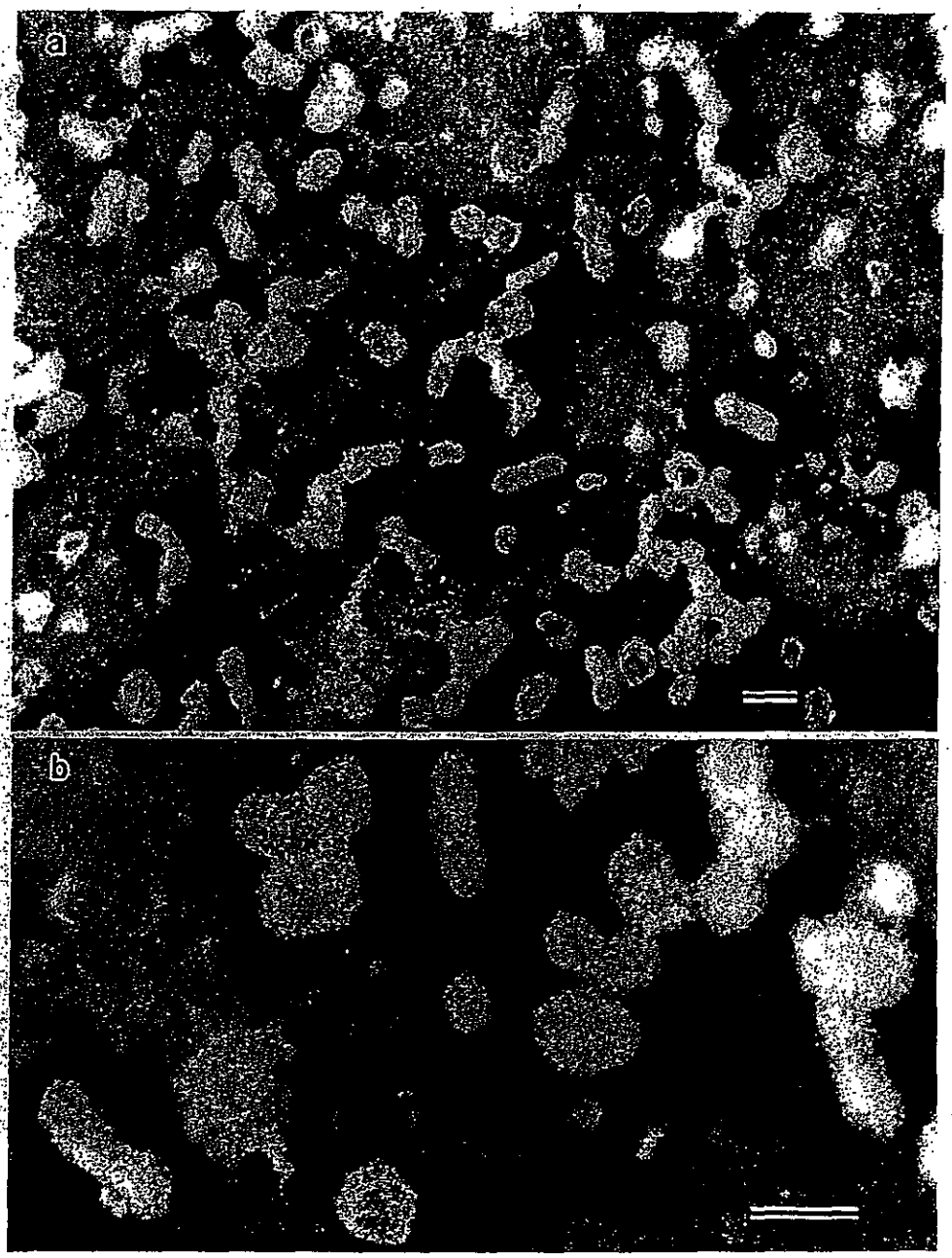

Fig. 2. Negatively stained electron micrograph of rodlike VLPs presented in plasma containing a moderate number of the spherical VLPs. Rodlike VLPs from sample no. 7 (a) and sample no. 5 (b) are shown. *Bar* 100 nm

sucrose density gradient centrifugation. The majority were 50- to 80-nm spherical particles with 9- to 12-nm-long surface projections (see Fig. 1), and were, if anything, morphologically resembling togaviruses or coronaviruses. Positive staining revealed that the electron-dense material combined with uranium existed in a 35- to 45-nm internal core structure of the spherical virus-like particle, and indicated that the spherical particles had the nucleic acid therein (see Fig. 3). In addition, rodlike VLPs (Fig. 2) were observed concomitantly in the specimens containing many spherical VLPs, and the surface spikelike projections of rodlike forms looked similar to those of the spherical particles. Interestingly, two internal cores (Fig. 3c) were detected in a rodlike form, suggesting that this form could be a diploid particle of the virus. Thus, we might consider that both the spherical and the rodlike virus-like particles belong to the same virus species.

In the past three decades, togavirus-like particles have been detected in acute-phase serum from a hemodialysed patient with non-A, non-B hepatitis and in the acute-phase urine of two icteric non-A, non-B hepatitis cases,[11] or in the liver of a patient with sporadic non-A, non-B fulminant hepatitis,[12] but the number of those objects was too small, and thus the etiological implications were not developed. Circulating VLPs, as presented here, were highly prevalent (92.3%) in ALT-elevated plasma samples, and were relatively easy to visualize by conventional electron microscopy, whereas in only one of ten normal controls were a few

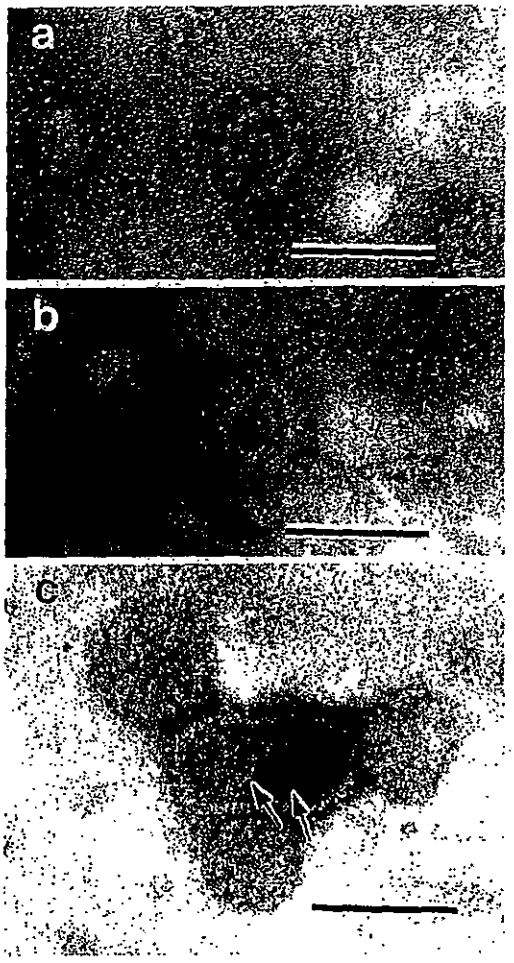

Fig. 3. Electron micrographs of positively stained VLPs showing internal corelike structures. Spherical VLPs from sample no. 6 (a) and sample no. 3 (b) and rodlike VLPs from sample no. 7 (c) are shown. a A 65-nm spherical VLP with a 35-nm inner core and surface spikelike projections; b 80-nm spherical VLP with a 45-nm core; c rodlike VLP 130 nm in the major axis and 70 nm in the minor axis containing two corelike structures (*arrows*) within an outer coat 10 nm thick. *Bar* 100 nm

spherical VLPs barely detectable despite careful observation. Thus, although the presence of the virus-like particles in the blood was significantly ($P < 0.001$) associated with the elevation of plasma ALT levels, indicating that these virus-like particles do cause liver cell necrosis, the nucleic acids of parenterally transmissible known hepatitis viruses were not detected in any tested plasma samples.

A novel DNA virus, which was designated TT virus (TTV), has been successfully cloned from serum of a patient with posttransfusion hepatitis of unknown etiology.[13] TTV particles are 30- to 32-nm spherical particles with a density of 1.31–1.35 g/ml in cesium chloride.[14] The morphology and buoyant density of TTV were quite different from the VLPs described in this article. Recently, a novel single-stranded DNA virus, which was named NV-F, has also been successfully cloned from the serum of a patient with non-A–E hepatitis without isolation of the virus particles.[15] NV-F DNA was detected in 17 (24.6%) of 69 patients with non-A–E and in 5 (2.8%) of 180 healthy individuals. Therefore, further morphological study and genomic study of NV-F should be carried out to evaluate whether the circulating VLPs were closely related to NV-F.

In conclusion, the presented VLPs, which morphologically resembled togaviruses or coronaviruses, may be a causative candidate virus of bloodborne non-A–G hepatitis, and details of the etiological implications should be further elucidated.

Acknowledgments The authors thank Drs. H. Okamoto and M. Mayumi, Immunology Division, Jichi Medical School, Tochigi, Japan, for testing the GBV-C genomic RNA. This study was supported in part by Grants-in Aid for Scientific Research (C) 17590632 from the Ministry of Education, Science and Culture of Japan.

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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	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.fda.gov/cber/faq/msmdonor.htm	公表国 米国	
販売名(企業名)	－					

研究報告の概要

男性間性交渉者(MSM)からの供血に関する米国食品医薬品局(FDA)の政策は、米国でAIDSが流行し始めた1977年以降に他の男性と性交渉を行ったことがある男性の供血は見合わせており、これは米国独自のものではなく、多くのヨーロッパ諸国も、この政策を維持しており、MSMからの供血永久停止を科学と倫理の両面から再検討している。
米国赤十字によると、1977年以降に男性間性交渉歴を持つ男性の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倍高いとされる。HIVに感染している男性間性交渉者の75%は、すでに自分がHIV陽性であることを自覚しており、供血する可能性は低いことを考慮に入れても、男性間性交渉歴を有する潜在的供血者のHIV有病率は、初回供血者よりも200倍高く、リピート供血者よりも2000倍高い。
現在の高感度検査がHIV感染供血者を検出できない割合は100万人中1人未満であるものの、米国で全血、赤血球濃縮製剤、血漿、血小板が輸血される件数は年間2000万件以上にのぼることに留意しなければならない。
非常に低レベルのウイルスが血中に存在する時期、いわゆる「ウインドウ期」では、HIV感染を検出することが特に難しい。
現在、輸血や血漿分画製剤からHIVが伝播するリスクは米国ではほぼ排除されている。
また、男性間性交渉者は、輸血により伝播され得る他の感染症を有するリスクも高い。例えば、男性間性交渉者は、一般集団よりも、B型肝炎ウイルス感染は約5～6倍多くみられ、C型肝炎ウイルス感染は約2倍多くみられる。さらに、男性間性交渉者の間でヒトヘルペスウイルス8型（HHV-8）の罹患率と有病率も高い。HHV-8は、免疫不全患者にカポジ肉腫と呼ばれる癌を引き起こす。

報告企業の意見	今後の対応
米国において実施されている、HIVをはじめとするウイルス疾患のハイリスクグループであるMSMからの供血制限に関する情報である。 血漿分画製剤では、採血時の問診、スクリーニング検査に加え、製造工程中において各種ウイルスの不活化・除去効果を有する工程が設けられている。	今後とも供血者からのHIV等の感染者排除に関する安全性情報等に留意していく。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

FDA Policy on Blood Donations from Men Who Have Sex with Other Men

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What is FDA's policy on blood donations from men who have sex with other men (MSM)?

Men who have had sex with other men, at any time since 1977 (the beginning of the AIDS epidemic in the United States) are currently deferred as blood donors. This is because MSM are, as a group, at increased risk for HIV, hepatitis B and certain other infections that can be transmitted by transfusion.

The policy is not unique to the United States. Many European countries have recently reexamined both the science and ethics of the lifetime MSM deferral, and have retained it (See the transcript of the "FDA Workshop on Behavior-Based Donor Deferrals in the NAT Era" at http://www.fda.gov/cber/minutes/nat030806t.htm#7 for further information.). This decision is also consistent with the prevailing interpretation of the European Union Directive 2004/33/EC article 2.1 on donor deferrals.

Why doesn't FDA allow men who have had sex with men to donate blood?

A history of male-to-male sex is associated with an increased risk for the presence of and transmission of certain infectious diseases, including HIV, the virus that causes AIDS. FDA's policy is intended to protect all people who receive blood transfusions from an increased risk of exposure to potentially infected blood and blood products.

The deferral for men who have had sex with men is based on the following considerations regarding risk of HIV:

- Men who have had sex with men since 1977 have an HIV prevalence (the total number of cases of a disease that are present in a population at a specific point in time) 60 times higher than the general population, 800 times higher than first time blood donors and 8000 times higher than repeat blood donors (American Red Cross). Even taking into account that 75% of HIV infected men who have sex with men already know they are HIV positive and would be unlikely to donate blood, the HIV prevalence in potential donors with history of male sex with males is 200 times higher than first time blood donors and 2000 times higher than repeat blood donors.
- Men who have had sex with men account for the largest single group of blood donors who are found HIV positive by blood donor testing.
- Blood donor testing using current advanced technologies has greatly reduced the risk of HIV transmission but cannot yet detect all infected donors or prevent all transmission by transfusions. While today's highly sensitive tests fail to detect less than one in a million HIV infected donors, it is important to remember that in the US there are over 20 million transfusions of blood, red cell concentrates, plasma or platelets every year. Therefore, even a failure rate of 1 in a million can be significant if there is an increased risk of undetected HIV in the blood donor population.
- Detection of HIV infection is particularly challenging when very low levels of virus are present in the blood for example during the so-called "window period". The "window period" is the time between being infected with HIV and the ability of an HIV test to detect HIV in an infected person.
- FDA's MSM policy reduces the likelihood that a person would unknowingly donate blood during the "window period" of infection. This is important because the rate of new infections in MSM is higher than in the general population and current blood donors.
- Collection of blood from persons with an increased risk of HIV infection also presents an added risk if blood were to be accidentally given to a patient in error either before testing is completed or following a positive test. Such medical errors occur very rarely, but given that there are over 20 million transfusions every year, in the USA, they can occur. That is one more reason why FDA and other regulatory authorities work to assure that there are multiple safeguards, not just testing.
- Several scientific models show there would be a small but definite increased risk to people who receive blood transfusions if FDA's MSM policy were changed and that preventable transfusion transmission of HIV could occur as a result.
- No alternate set of donor eligibility criteria (even including practice of safe sex or a low number of lifetime partners) has yet been found to reliably identify MSM who are not at increased risk for HIV or certain other transfusion

transmissible infections.

- Today, the risk of getting HIV from a transfusion or a blood product has been nearly eliminated in the United States. Improved procedures, donor screening for risk of infection and laboratory testing for evidence of HIV infection have made the United States blood supply safer than ever. While appreciative and supportive of the desire of potential blood donors to contribute to the health of others, FDA's first obligation is to assure the safety of the blood supply and protect the health of blood recipients.
- Men who have sex with men also have an increased risk of having other infections that can be transmitted to others by blood transfusion. For example, infection with the Hepatitis B virus is about 5-6 times more common and Hepatitis C virus infections are about 2 times more common in men who have sex with other men than in the general population. Additionally, men who have sex with men have an increased incidence and prevalence of Human Herpes Virus-8 (HHV-8). HHV-8 causes a cancer called Kaposi's sarcoma in immunocompromised individuals.

What is self-deferral?

Self-deferral is a process in which individuals elect not to donate because they identify themselves as having characteristics that place them at potentially higher risk of carrying a transfusion transmissible disease. FDA uses self-deferral as part of a system to protect the blood supply. This system starts by informing donors about the risk of transmitting infectious diseases. Then, potential donors are asked questions about their health and certain behaviors and other factors (like travel and past transfusions) that increase their risk of infection. Screening questions help people, even those who feel well, to identify themselves as potentially at higher risk for transmitting infectious diseases. Screening questions allow individuals to self defer, rather than unknowingly donating blood that may be infected.

Is FDA's policy of excluding MSM blood donors discriminatory?

FDA's deferral policy is based on the documented increased risk of certain transfusion transmissible infections, such as HIV, associated with male-to-male sex and is not based on any judgment concerning the donor's sexual orientation.

Male to male sex has been associated with an increased risk of HIV infection at least since 1977. Surveillance data from the Centers for Disease Control and Prevention indicate that men who have sex with men and would be likely to donate have a HIV prevalence that is at present over 15 fold higher than the general population, and over 2000 fold higher than current repeat blood donors (i.e., those who have been negatively screened and tested) in the USA. MSM continue to account for the largest number of people newly infected with HIV.

Men who have sex with men also have an increased risk of having other infections that can be transmitted to others by blood transfusion.

What about men who have had a low number of partners, practice safe sex, or who are currently in monogamous relationships?

Having had a low number of partners is known to decrease the risk of HIV infection. However, to date, no donor eligibility questions have been shown to reliably identify a subset of MSM (e.g., based on monogamy or safe sexual practices) who do not still have a substantially increased rate of HIV infection compared to the general population or currently accepted blood donors. In the future, improved questionnaires may be helpful to better select safe donors, but this cannot be assumed without evidence.

Are there other donors who have increased risks of HIV or other infections who, as a result, are also excluded from donating blood?

Intravenous drug abusers are excluded from giving blood because they have prevalence rates of HIV, HBV, HCV and HTLV that are much higher than the general population. People who have received transplants of animal tissue or organs are excluded from giving blood because of the still largely unknown risks of transmitting unknown or emerging pathogens harbored by the animal donors. People who have recently traveled to or lived abroad in certain countries may be excluded because they are at risk for transmitting agents such as malaria or variant Creutzfeldt-Jakob Disease (vCJD). People who have engaged in sex in return for money or drugs are also excluded because they are at increased risk for transmitting HIV and other blood-borne infections.

Why are some people, such as heterosexuals with multiple partners, allowed to donate blood despite increased risk for transmitting HIV and hepatitis?

Current scientific data from the U.S. Centers for Disease Control and Prevention (CDC) indicate that, as a group, men who have sex with other men are at a higher risk for transmitting infectious diseases or HIV than are individuals in other risk categories. While statistics indicate a rising infection rate among young heterosexual women, their overall rate of HIV infection remains much lower than in men who have sex with other men. For information on HIV-related statistics and trends, go to CDC's HIV/AIDS Statistics and Surveillance web page.

Isn't the HIV test accurate enough to identify all HIV positive blood donors?

HIV tests currently in use are highly accurate, but still cannot detect HIV 100% of the time. It is estimated that the HIV risk from a unit of blood has been reduced to about 1 per 2 million in the USA, almost exclusively from so called "window period" donations. The "window period" exists very early after infection, where even current HIV testing methods cannot detect all infections. During this time, a person is infected with HIV, but may not have made enough virus or developed enough antibodies to be detected by available tests. For this reason, a person could test negative, even when they are actually HIV positive and infectious. Therefore, blood donors are not only tested but are also asked questions about

behaviors that increase their risk of HIV infection.

Collection of blood from persons with an increased risk of HIV infection also presents an added risk to transfusion recipients due to the possibility that blood may be accidentally given to a patient in error either before testing is completed or following a positive test. Such medical errors occur very rarely, but given that there are over 20 million transfusions every year, in the USA, they can occur. For these reasons, FDA uses a multi-layered approach to blood safety including pre-donation deferral of potential donors based on risk behaviors and then screening of the donated blood with sensitive tests for infectious agents such as HIV-1, HIV-2, HCV, HBV and HTLV-I/II.

How long has FDA had this MSM policy?

FDA's policies on donor deferral for history of male sex with males date back to 1983, when the risk of AIDS from transfusion was first recognized. Our current policy has been in place since 1992.

FDA has modified its blood donor policy as new scientific data and more accurate tests for HIV and hepatitis became available. Today, the risk of getting HIV from a blood transfusion has been reduced to about one per two million units of blood transfused. The risk of hepatitis C is about the same as for HIV, while the risk of hepatitis B is somewhat higher.

Doesn't the policy eliminate healthy donors at a time when more donors are needed because of blood shortages?

FDA realizes that this policy will defer many healthy donors. However, FDA's MSM policy minimizes even the small risk of getting infectious diseases such as HIV or hepatitis through a blood transfusion.

Would FDA ever consider changing the policy?

FDA scientists continue to monitor the scientific literature and to consult with experts in CDC, NIH and other agencies. FDA will continue to publicly revisit the current deferral policy as new information becomes available.

On March 8, 2006, FDA conducted a workshop entitled "Behavior-based donor deferrals in the Nucleic Acid Test (NAT) era". The workshop addressed scientific challenges, opportunities, and risk based donor deferral policies relevant to the protection of the blood supply from transfusion transmissible diseases, seeking input on this topic. Participants were given the opportunity to provide scientific data that could support revising FDA's MSM deferral. The workshop provided a very active, open and broad-based scientific dialogue concerning current behavior-based deferrals and explored other options that may be considered and the data needed to evaluate them.

FDA's primary responsibility is to enhance blood safety and protect blood recipients. Therefore FDA would change this policy only if supported by scientific data showing that a change in policy would not present a significant and preventable risk to blood recipients. Scientific evidence has not yet been provided to FDA that shows that blood donated by MSM or a subgroup of these potential donors, is as safe as blood from currently accepted donors.

FDA remains willing to consider new approaches to donor screening and testing, provided those approaches assure that blood recipients are not placed at an increased risk of HIV or other transfusion transmitted diseases.

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Updated: May 23, 2007

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. 3. 19	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)		Chen YM, Kuo SH. Lancet. 2007 Feb 24;369(9562):623-5.	台湾

研究報告の概要

○台湾のHIV-1

台湾のHIV-1/AIDS感染拡大は危険な状況に突入しつつある。2006年末までに外国人599名を含む13,702名のHIV-1感染が台湾の疾病対策センター(CDC)に報告された。2003年の初回供血者、徴集兵、妊婦におけるHIV-1感染率は、それぞれ10万人当り5.2人、57.0人、12.0人であった。同年のHIV-1感染率は、静注薬物使用者(IDU)で0.09%、女性風俗従業員で0.2%、性感染症患者で1.9%、男性と性交渉を持つ男性(MSM)で6.7%であった。感染者数は2003年に11%増、2004年に77%増、2005年に123%増と急増したが、感染拡大予防プログラム実施後の2006年には10%減少した。最近の推定では台湾のHIV-1/AIDS感染者数は約3万人で、感染率(2,300万人中3万人;1/767)は中国(13億人中65万人;1/2,000)よりも高い可能性が示されている。

リスク要因分析によると、IDUのHIV-1感染率は、2002年の1.7% (13/772)から2003年の8.1%(70/862)、2004年の41.3%(628/1,520)、2005年の72.4% (2,461/3,399)へと増加し、2006年には68.6% (2,017/2,974)に減少した。台湾のIDU6万～10万人のうち、10～15%はHIV-1のCRF07_BC株に感染していると推定される。

同性愛者用サウナを利用するMSMのHIV-1感染率は5.2%～15.8%である。MSMのHIV-1/AIDS感染者は、異性愛者と比べて梅毒の有病率も有意に高い。HIV-1/AIDS感染者のうち20歳未満の割合は、異性愛者(1.7%)と比較してMSM(3.0%)では有意に多い。

HIV-1の垂直感染例は、2006年末までに確定例19例が報告された。台湾CDCは、2005年1月に母子感染予防プログラムを開始し、2005年中に5例の垂直感染が報告された。2006年6月までにスクリーニング率は97.4%に達し、妊婦338,452名中47名(10万人当り13.9人)の感染が特定され、母子感染予防のための抗レトロウイルス療法を受けた。

台湾でHIV-1感染の脅威が高まるにつれ、根強い感染の否定、差別再燃の兆候が多くみうけられる。

報告企業の意見	今後の対応
2003年以降急増した台湾のHIV-1/AIDS感染者数は約3万人と推測され、感染率は中国よりも高い可能性が示され危険な状態に入りつつあるとの報告である。	日本赤十字社では、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。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

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HIV-1 in Taiwan

Taiwan is entering a new and dangerous phase of its HIV-1/AIDS epidemic. By the end of 2006, 13 702 individuals (including 599 foreigners) had been reported as infected with HIV-1 to the Centers for Disease Control of Taiwan.[1] In 2003, HIV-1 rates in first-time blood donors, military conscripts, and pregnant women were measured at 5·2, 57·0, and 12·0 per 100 000, respectively.[1] Data from that year indicated HIV-1 rates of 0·09% for intravenous drug users, 0·2% for female sex workers, 1·9% for patients with sexually transmitted infections, and 6·7% for men who have sex with men in saunas or bath houses.[1] Since then, the number of people living with HIV-1/AIDS in Taiwan has jumped sharply, from an 11% increase in 2003 to a 77% increase in 2004 and a 123% increase in 2005 (figure 1).[1]

However, after the implementation of a harm-reduction programme, a 10% decrease was seen in 2006 (figure 1). The current estimated number of HIV-1/AIDS cases in Taiwan is about 30 000, which suggests that the infection rate there could be greater than that in China: 30 000 per 23 million (1/767) compared with 650 000 per 1·3 billion (1/2000).[2]

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

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Of the 60 000–100 000 intravenous drug users in Taiwan, 10–15% may be infected with CRF07_BC. If so, they probably represent the largest group of such intravenous drug users in northeast Asia. The circulating recombinant form might have followed a separate drug-trafficking route to Taiwan from Yunnan

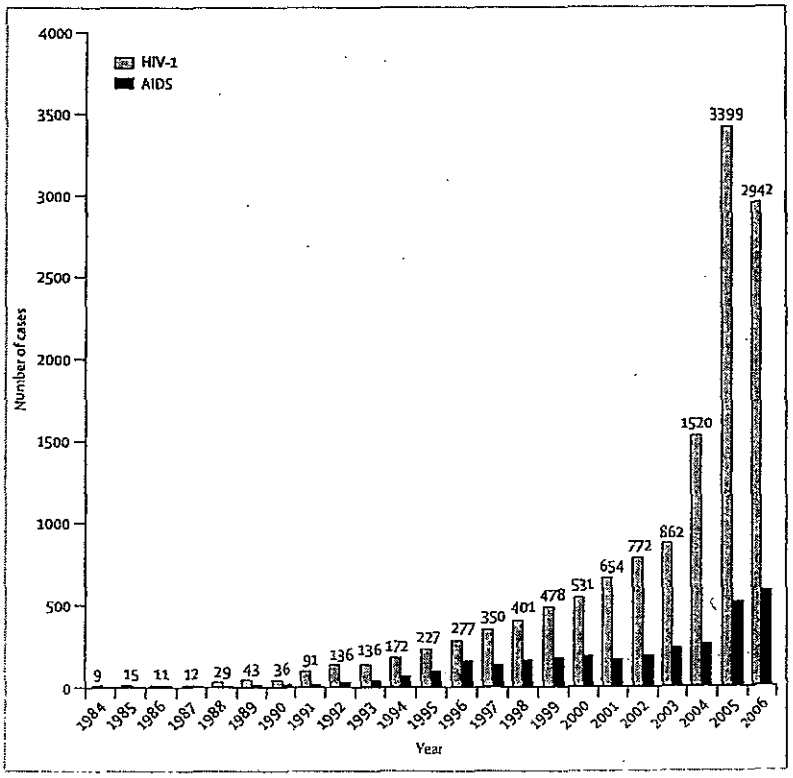

Figure 1: Annual numbers of HIV-1 seropositive cases and AIDS patients reported to Taiwan Centers for Disease Control[1]

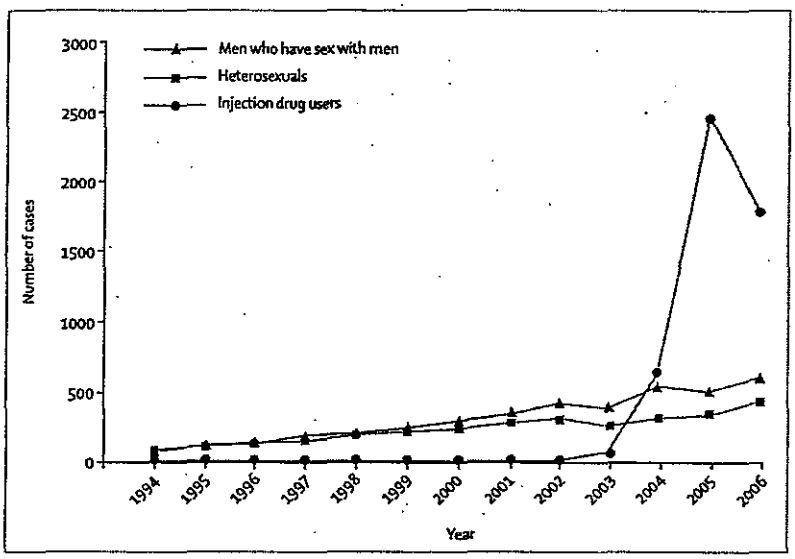

Figure 2: Annual numbers of HIV-1-infected persons in various high-risk groups reported to Taiwan Centers for Disease Control[1]

via southeast China, Guangxi province, and Hong Kong.[7-9] There have been enormous increases in the amount of heroin smuggled into Taiwan and in the number of intravenous drug users since 2002, when five intravenous drug users from southern Taiwan were diagnosed as the country's first HIV-1 seropositive cases infected with CRF07_BC.[5] Even though the Hong Kong authorities identified three cases of CRF07_BC infection in 2001, a serious outbreak in that city's population of intravenous drug users is believed to have been blocked by a methadone maintenance programme.[9]

Clearly, close monitoring of emerging HIV-1 subtypes related to intravenous drug use and implementing harm-reduction programmes are vital to preventing similar outbreaks in other populations of intravenous drug users in neighbouring countries. In 2005, Alex Wodak, Jerry Stimson, and other harm-reduction experts were invited to Taiwan to share their experiences with government officials, medical field-workers, and public-health professionals. After careful study of harm-reduction programmes in place in Hong Kong and Australia, a pilot programme was started in four of Taiwan's 23 administrative areas in September, 2005. This programme has since been expanded nationally, and consists of 427 service sites for syringe exchange plus centres for methadone maintenance therapy. Free methadone is provided to HIV-1-infected intravenous drug users while HIV-1 seronegative intravenous drug users have to pay about US$1600 a year. The Taiwan Centers for Disease Control plans to provide methadone maintenance to intravenous drug users in prisons, and the country's Bureau of Controlled Drugs will start producing methadone to assist in the government's commitment to providing methadone maintenance to 30 000 intravenous drug users by 2009.

All parts of Asia are reporting rising numbers of HIV-positive and AIDS patients in male homosexuals and bisexuals. In Taiwan, HIV-1 infection rates in men who have sex with men in gay saunas in different cities currently range from 5·2% to 15·8%.[10,11] The same population has high rates of syphilis, 8·1–13·8%, depending on the city.[10,11] Taiwanese male homosexual and bisexual HIV-1/AIDS patients have also been diagnosed with significantly higher rates of syphilis than have heterosexual patients.[12] Furthermore, the percentage of homosexual or bisexual HIV-1/AIDS patients under the age of 20 years is significantly higher than that of heterosexual patients, 3·0% versus 1·7%.[12] In addition to the stigmatisation of homosexuality in Taiwanese society, the lack of accurate information on homosexuality in sex education and on risk factors in AIDS education increases the risk of contracting HIV and other sexually transmitted infections within the country's population of men who have sex with men. Whilst a community-based prevention programme for such men has been developed by a group of academic and grass-roots non-governmental organisations, a current challenge is the implementation of this programme into a national programme, and making it a priority.

Taiwan's clinical spectrum of AIDS patients is similar to those reported in other developed countries, but significant differences have been noted in incidences of opportunistic infections. For example, the incidence of tuberculosis in patients with advanced illness is high in Taiwan (24·6%) and the rate of endemic fungal (*Penicillium marneffei*) infections is increasing.[13,14] On the positive side, the effort by the Taiwanese Government since April, 1997, to distribute highly-active antiretroviral therapy for free[15] has resulted in dramatic decreases in morbidity and mortality from HIV-1 infection.[16]

Because of their high background prevalence, HBV and HCV coinfections with HIV are particularly important in Asian countries in terms of HIV transmission via injecting drug use.[17,18] In a survey of

459 intravenous drug users infected with HIV-1, one of us (Y-MAC) found that 456 (99·6%) also had anti-HCV antibodies and 77 (16·8%) were seropositive for HBsAg. The long-term impact of hepatitis coinfections on HIV and on morbidity and mortality from liver disease requires monitoring.

By the end of 2006, 19 confirmed cases of vertical HIV-1 transmission have been reported to the Taiwan Centers for Disease Control.[1] In January, 2005, the agency started a national programme focused on prevention of mother-to-child transmission, and five cases of vertical transmission were reported in 2005. By June, 2006, the screening rate had reached 97·4%, and 47 of 338 452 pregnant women (13·9 per 100 000) tested in Taiwan have been identified as having HIV-1 infections and have received antiretroviral therapy to prevent mother-to-child transmission. To increase the participation rate, there is discussion of changing the voluntary counselling and testing strategy from opt in to opt out.

Several positive responses to the HIV/AIDS epidemic in Taiwan should be mentioned. In 1990 an AIDS Prevention and Control Law was passed to protect the rights of people with HIV/AIDS for treatment, education, and employment. Since 1992, 16 non-governmental organisations registered or established in Taiwan have provided shelter, care, counselling, anonymous testing, and AIDS education. One in particular, the People Living with HIV/AIDS Rights' Advocacy Association, has been addressing human rights issues related to HIV/AIDS since 1997. However, most such organisations have their headquarters and facilities in northern Taiwan, and two-thirds of the country's intravenous drug users live in central and southern parts. In addition, many social workers employed by non-governmental organisations are still unfamiliar with issues related to drug abuse and inexperienced in interacting with intravenous drug users. There is a clear and immediate need for counselling workshops for medical staff and social workers.

As the HIV-1 infection threat increases, there are many signs of persistent denial and resurgent discrimination in Taiwan. Several important issues need to be addressed: sentinel surveillance of female sex workers, social welfare institutions and housing for homeless people with HIV/AIDS, financial support for non-governmental organisations, training and re-education programmes aimed at changing the attitudes of medical staff toward people with HIV/AIDS, and more funding for AIDS research, especially vaccine development.

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We declare that we have no conflict of interest.

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別紙様式第2-1
番号18

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007年4月11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	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	第81回日本感染症学会総会・学術講演会 ポスターP26-1	公表国 日本	
販売名（企業名）	①テタノブリンーIH（ベネシス） ②テタノブリン（ベネシス）					

研究報告の概要

【緒言】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感染症例を経験したので報告する。

【症例】77歳男性、36年前セネガルで輸血歴がある。2006年6月下旬、気管支喘息発作にて当院入院となった。インフォームド・コンセントの上での入院時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法により確認検査を行い、HIV-1抗体陰性HIV-2抗体陽性となった。また、ペプチド法による確認検査でも同様の結果であった。入院時のCD4数は234/μLとやや低値であったがAIDSを疑わせる症状は認められなかった。加療にて気管支喘息は軽快し入院8日目で退院となった。8月現在CD4数は827/μLとなりAIDSを発症せずに当院外来で経過観察中である。

【遺伝子解析】国立感染症研究所に依頼し、HIV-1及びHIV-2各々に特異的なgag及びnef-LTR領域を標的とするプライマーを用いたPCRによる遺伝子検査を行った、その結果、HIV-2特異的gagプライマーでのみプロウイルスDNAの増幅が確認された。更にPCR産物から得られた塩基配列の系統樹解析では、本症例は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。

【考察】輸血歴と遺伝子解析の結果から、本症例は36年前セネガルでの輸血でHIV-2に感染したと考えられる。HIV-2は一般的に発症が遅く症状が軽いとされているが、本症例が36年間AIDSを発症していない機序は極めて興味深く、現在国立感染症研究所と共同で調査中である。なお、国内におけるHIV-2感染は稀とはいえ、HIVスクリーニング検査陽性でHIV-1感染に特異的な検査が陰性である場合、HIV-2感染の可能性を考慮する必要性がある。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリンーIHの記載を示す。

2. 重要な基本的注意

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

報告企業の意見	今後の対応
日本人初のHIV-2感染者が確認されたとの報告である。 万一、原料血漿にHIV-2が混入したとしても、HIV-1をモデルウイルスとしたウイルスバリデーション試験成績から、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。

(5)

ポスター26　HIV感染症 1

G0701500

P26-1　36年間AIDSを発症していない日本人初のHIV-2感染症の1例

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【緒言】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感染症例を経験したので報告する．【症例】77歳男性．36年前セネガルで輸血歴がある．2006年6月下旬，気管支喘息発作にて当院入院となった．インフォームド・コンセントの上での入院時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法により確認検査を行い，HIV-1抗体陰性HIV-2抗体陽性となった．また，ペプチド法による確認検査でも同様の結果であった．入院時のCD4数は234/μLとやや低値であったがAIDSを疑わせる症状は認められなかった．加療にて気管支喘息は軽快し入院8日目で退院となった．8月現在CD4数は827/μLとなりAIDSを発症せずに当院外来で経過観察中である．【遺伝子解析】国立感染症研究所に依頼し，HIV-1及びHIV-2各々に特異的な*gag*及び*nef*-LTR領域を標的とするプライマーを用いたPCRによる遺伝子検査を行った．その結果，HIV-2特異的*gag*プライマーでのみプロウイルスDNAの増幅が確認された．更にPCR産物から得られた塩基配列の系統樹解析では，本症例は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．【考察】輸血歴と遺伝子解析の結果から，本症例は36年前セネガルでの輸血でHIV-2に感染したと考えられる．HIV-2は一般的に発症が遅く症状が軽いとされているが，本症例が36年間AIDSを発症していない機序は極めて興味深く，現在国立感染症研究所と共同で調査中である．尚，国内におけるHIV-2感染は稀とはいえHIVスクリーニング検査陽性でHIV-1感染に特異的な検査が陰性である場合，HIV-2感染の可能性を考慮する必要性がある．（会員外共同研究者：草川茂[2]，上西理恵[2]）

G0701501

P26-2　初回治療における硫酸アタザナビルの使用経験

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【目的】硫酸アタザナビル（ATV）はHIVプロテアーゼ阻害作用を有し，HIV感染症に用いられる薬剤である．本剤は1日1回投与の適応を持ち，服薬アドヒアランスの向上が期待できることから，治療の第一選択薬の一つとして使用されている薬剤である．今回我々は，ATV服用患者を対象に，治療効果・安全性について検討を行ったので報告する．【方法】平成16年6月から平成18年5月までに，当院で本剤の投薬を開始した未治療患者60例を対象に調査を行った．【結果】対象患者60例中，核酸系逆転写酵素阻害剤（NRTI）2剤にATV 400mgを併用した症例は7例，NRTI 2剤にATV 300mgとリトナビル（RTV）100 mgを併用した症例は53例であった．NRTIの主な併用薬はTDF＋3TC 24例，TDF＋FTC 23例であった．抗ウイルス効果について24週以上投与された症例で検討した．投薬開始後4週を経過した時点のHIV-RNA量は，平均1.9log10copies/ml減少し，24週，48週後にHIV-RNA量が検出限界未満（50copies/ml）であった症例は，それぞれ45/47，36/36であった．主な副作用は「総ビリルビン上昇」「黄疸」「黄疸眼」であったが，その多くは軽度であり，副作用が原因で他剤への変更が行われた症例は1例であった．総コレステロール（TC），中性脂肪（TG）の変化を投与前と投与24週，48週後で検討した．TCの変化率は，＋1.1%，＋1.1%，TGは，＋1.3%，＋1.1%であった．【考察】一般的にPIは脂質代謝への影響が大きく，長期服用が必要とされる抗HIV療法の問題の一つとされているが，本剤はTC，TGへの影響が少ない薬剤であると考えられた．ATVは抗ウイルス効果に優れ特に問題となる副作用も認められないことから，認容性の高いPIである思われた．

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. 4. 19	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Hamaguchi T, Noguchi-Shinohara M, Nakamura Y, Sato T, Kitamoto T, Mizusawa H, Yamada M. Emerg Infect Dis. 2007 Jan;13(1):162-4.	公表国
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○日本のプリオン疾患における眼科手術

孤発性クロイツフェルト・ヤコブ病患者のうち10%～20%は、疾患の早期の段階で視覚障害を発症する。一部の患者は、プリオン疾患あるいは加齢による視覚障害のために眼科を受診する。手術後長期間経ってからプリオン疾患を発症した場合、眼科手術による感染性プリオンタンパクの二次感染予防は困難である。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。

報告企業の意見	今後の対応
日本のプリオン疾患患者597名のうち11名が、発症の前後1ヶ月以内に眼科手術を受け、眼科医はプリオンタンパク質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との報告である。	今後も引き続き、プリオン病に関する新たな知見及び情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

Ophthalmic Surgery in Prion Diseases

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Eleven (1.8%) of 597 patients underwent ophthalmic surgery within 1 month before the onset of prion disease or after the onset. All ophthalmologists reused surgical instruments that had been incompletely sterilized to eliminate infectious prion protein. Ophthalmologists should be aware of prion diseases as a possible cause of visual symptoms and use disposable instruments whenever possible.

Visual impairment occurs in 10% to 20% of patients with sporadic Creutzfeldt-Jakob disease (sCJD) during an early stage of the disease (Heidenhain variant) (*1*,*2*). Some patients with prion diseases may visit ophthalmologists with visual impairment due to prion diseases or with coexisting age-related eye diseases (*3*,*4*).

Infectious prion protein (PrP^{Sc}) was identified in the retina and optic nerve in patients with variant CJD (vCJD) and sCJD (*5*,*6*), and CJD has been transmitted by corneal transplantation (*7*,*8*). In the World Health Organization (WHO) guidelines, eyes were classified as highly infectious tissues (*9*).

Secondary transmission of PrP^{Sc} through ophthalmic surgery could possibly be prevented around the onset of prion diseases, although surgery that is performed long before the onset of prion diseases would not have that potential. It is important to understand the current status of ophthalmic surgery for patients with prion diseases and to clarify the clinical features of the patients with prion diseases who undergo ophthalmic surgery. Here, we describe the relevant data from CJD surveillance in Japan.

The Study

We analyzed the patients with prion diseases who had been registered by the CJD Surveillance Committee in Japan from April 1999 through March 2005. We prospectively investigated each patient with a surveillance protocol that assembled information about life history, previous medical history, clinical history, laboratory data, and results of molecular genetic and pathologic analyses. Written consent, approved by the Institutional Ethics Committee, was obtained from all the patients' families; members of the Surveillance Committee examined the patients and collected the data.

We classified the patients into 4 categories: sCJD, infectious prion diseases, inherited prion diseases, and unclassified prion diseases. sCJD was diagnosed according to the classical criteria established by Masters et al. (*10*). Infectious prion diseases included CJD associated with cadaveric dura mater graft (dCJD) or other iatrogenic opportunities for prion infection, in which the criteria for sCJD were applied for the diagnosis, and vCJD, in which the diagnosis was based on WHO criteria (2001) (*11*). Regarding the accuracy of the diagnosis of inherited prion diseases, cases verified by pathology report were defined as definite, and cases with mutations in the prion protein gene and neuropsychiatric manifestations compatible with prion diseases were defined as probable.

Among patients with a history of ophthalmic surgery, we directed special attention to the patients who had a history of eye surgery within 1 month before the obvious onset of prion disease or after the onset. Because the onset of prion diseases often overlaps with various kinds of prodromal symptoms, determining the precise time point of onset is difficult; therefore, we included the period of 1 month before the obvious onset. To gather information about the ophthalmic surgery, we mailed questionnaires to the ophthalmologists who operated on these patients, requesting the following information: diagnosis of ophthalmologic diseases, surgical procedures performed, changes in the symptoms after the surgery, whether the instruments were reused, and methods of cleaning reused instruments.

To ascertain the clinical features of prion diseases, we analyzed the patient's age at onset and duration of disease course, which was calculated as the interval between the onset and the appearance of the akinetic mutism state or death in the patients who died without akinetic mutism. Among early clinical manifestations of prion diseases, dementia and visual disturbance are major determinants that would influence the indication for ophthalmic surgery, so we grouped the patients according to whether they had dementia or visual impairment within 2 months after onset of symptoms.

The sex distribution of the patients who had ophthalmic surgery around the time of onset of clinical symp-

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toms and those who did not was compared by Fisher exact tests, and differences in age at onset and disease duration were compared by Mann-Whitney U tests. We used χ^2 tests to compare the distribution of the patients with or without dementia or visual impairment within 2 months of onset. Statistical significance was defined as $p<0.05$.

We found 597 patients with definite or probable diagnosis of prion diseases: 468 (78.4%) with sCJD; 78 (13.1%) with inherited prion diseases; 48 (8.0%) with infectious prion diseases, including 47 cases of dCJD; and 1 patient with vCJD and 3 patients with unclassified CJD.

Thirty-seven patients (6.2%) had a history of ophthalmic surgery at some time in their lives. Among them, 11 patients (1.8%) underwent ophthalmic surgery within 1 month before the obvious onset of prion disease or after the onset. Except for 1 patient with Gerstmann-Sträussler-Scheinker disease, all of these patients had sCJD. There have been no reports of the development of prion diseases in patients who underwent ophthalmic surgery after the ophthalmic surgery of patients with prion diseases.

Ten patients with sCJD underwent ophthalmic surgery within 14 months of symptom onset, and 8 of them had ophthalmic surgery within 4 months of symptom onset (Table 1). At clinical onset, 4 patients exhibited visual symptoms, 5 had dementia, and 1 patient had a gait disturbance. All patients underwent surgery for cataracts, except for 1 patient who underwent surgery for a detached retina. According to the reports on the surgical outcome by the ophthalmologists of 7 patients, visual disturbance was unchanged in 2 patients, deteriorated in 1, and improved to some extent in 4 after surgery. All ophthalmologists reused some surgical instruments and cleaned instruments by either autoclaving or the ethylene oxide gas method, which have been reported to incompletely sterilize PrP^{Sc} (*9,12*).

Clinical features were compared between sCJD patients who did and did not have ophthalmic surgery (Table 2). The patients who had ophthalmic surgery had a significantly longer disease duration than those without ($p=0.0004$). Regarding early clinical symptoms within 2 months after onset, the subgroup with visual symptoms without dementia was significantly overrepresented among the patients who had ophthalmic surgery compared with those who did not have surgery ($p= 0.0004$).

Conclusions

Our study showed that, in 1.8% of the patients with prion diseases, eye tissues were operated on within 1 month before the obvious onset of prion disease or after the onset. In addition, the sCJD patients who underwent surgery had a significantly longer duration of the disease course as well as significant overrepresentation of visual symptoms without dementia in the early phase, compared with patients who did not have ophthalmic surgery.

The prevalence of ophthalmic surgery around the time of clinical onset of prion diseases in our study is similar to that (2.0%) in a report from the United Kingdom (*13*). In the UK study (*13*), patients with Heidenhain variant cases constituted 40% of sCJD patients who had ophthalmic surgery. Early visual impairment (due to prion diseases) would prompt ophthalmologists to perform surgery.

Currently, cataract surgery is recommended to improve physical or cognitive function in elderly patients (*14,15*). It should be noted that, after performing eye surgery on patients with prion disease, all ophthalmologists reused surgical instruments that were sterilized with procedures that are incomplete for the sterilization of PrP^{Sc}, although the WHO infection control guidelines for prion diseases (*9*) strongly recommend single-use surgical

Table 1. Characteristics of sCJD patients and ophthalmic surgery*

Patient no.	Sex/age, y†	Disease duration, mo‡	Symptom at sCJD onset	Ophthalmic disease	Interval, mo§	Visual symptoms after surgery	Reused instruments	Cleaning method
1	M/81	8	Visual	Cataract	4	NA	NA	NA
2	M/61	15	Dementia	Cataract	0	Improved	Yes	Autoclave (135°C for 9 min)
3	F/64	20	Visual	Cataract	14	Not changed	Yes	EOG
4	F/59	3	Dementia	Detached retina	–1	Improved	Yes	EOG
5	F/57	10	Dementia	Cataract	10	NA	NA	NA
6	F/79	5	Dementia	Cataract	–1	Improved	Yes	EOG
7	M/74	16	Visual	Cataract	3	Improved	Yes	Autoclave (132°C for 10 min), EOG
8	F/63	5	Visual	Cataract	1	Deteriorated	Yes	Autoclave (132°C for 10 min)
9	M/79	6	Gait disturbance	Cataract	2	Not changed	Yes	Autoclave (121°C for 60 min)
10	F/66	3	Dementia	Cataract	1	NA	NA	NA

*sCJD, sporadic Creutzfeldt-Jakob disease; visual, visual impairment; NA, not available; EOG, ethylene oxide gas.
†At sCJD onset.
‡Disease duration, the duration from onset to akinetic mutism state or death if the patients never displayed akinetic mutism.
§Between surgery and sCJD symptoms.

Table 2. Clinical symptoms of sCJD within 2 mo after disease onset*

Characteristic	Ophthalmic surgery		Total	p value
	No, n = 458	Yes, n = 10		
Female/male	263/195	6/4	269/199	0.57
Age at onset, y; mean ± SD	66.8 ± 9.9	68.3 ± 9.1	66.8 ± 9.9	0.74
Disease duration,† mean ± SD	4.2 ± 4.8	9.1 ± 6.0	4.3 ± 4.9	0.0004
Clinical symptoms (%)				
Dementia (+)/visual impairment (+)	153 (34.2)	4 (40.0)	157 (34.3)	
Dementia (+)/visual impairment (–)	239 (53.3)	3 (30.0)	242 (52.8)	0.0004
Dementia (–)/visual impairment (+)	16 (3.6)	3 (30.0)	19 (4.1)	
Dementia (–)/visual impairment (–)	40 (8.9)	0	40 (8.7)	

*sCJD, sporadic Creutzfeldt-Jakob disease; SD, standard deviation; +, with; –, without.
†Disease duration, the duration from onset to akinetic mutism or death if patients never displayed akinetic mutism.

instruments for procedures involving highly infective tissues. The fact that no secondary iatrogenic cases that could be attributed to surgical procedures were found during our investigation does not diminish the need for ophthalmologists to be aware of CJD as a cause of visual symptoms (including symptoms mimicking those of cataracts) and highlight the importance of using disposable instruments whenever possible to avoid cross-contamination.

Acknowledgments

We thank Fumio Moriwaka, Yoshiyuki Kuroiwa, Masatoyo Nishizawa, Nobuyuki Sodeyama, Masatoshi Takeda, Yusei Shiga, Shigetoshi Kuroda, Shigeki Kuzuhara, Jun Tateishi, Hiroyuki Murai, and Shigeo Murayama for the CJD surveillance.

The CJD Surveillance Committee belongs to the Research Group on Prion Disease and Slow Virus Infection, funded by the Ministry of Health, Labour and Welfare, Japan; the funding source had no involvement in the publication of this article.

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別紙様式第2-1

No. 12

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. 5. 22	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況	ABC Newsletter. 2007 May 4.	公表国 米国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)			

研究報告の概要

○イスラエルはvCJD及び肝炎に関する供血延期基準を変更
イスラエルで血液事業を行っている赤盾ダビデ社(Magen David Adom:MDA)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。イギリスでウシ海綿状脳症(「狂牛病」)の流行が始まった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、引き続き供血延期となる。vCJDの発生リスクはイギリスで600/100万、アイルランドで17/100万、ポルトガルで20/100万であるのに対し、フランスではわずかに1.7/100万であり、リスク要因としてはあまりにも小さい。このため、MDAは(保健省の承認を得て)フランス系移民及び旅行者に対し制限を緩和することを決定した。
加えてMDAは、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、胃や小腸の生検を含む内視鏡検査を受けた人の供血延期期間を1年から6ヵ月に短縮した(内視鏡検査を受けた人の供血延期は、生検に使用された内視鏡が完全に滅菌されずに再使用された場合、ウイルス感染症やvCJDを伝播しうるとの理論的可能性による)。
また、動物に噛まれた人は、噛んだ動物が不明であったり検査を受けていない場合、これまでの12ヵ月後ではなく2ヵ月後から供血が可能となる。

使用上の注意記載状況・その他参考事項等

白血球除去赤血球「日赤」
照射白血球除去赤血球「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
イスラエルで血液事業を行っている赤盾ダビデ社は変異型クロイツフェルト・ヤコブ病に関する供血延期基準を変更し、1980年以降フランスに居住歴がある人の供血を可能にしたとの報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

On the Difference between What People Say and What They Do About Risk

"If you ask me based on findings, (if people are) afraid of food recalls, the answer is no. So people aren't really concerned or scared, but the funny thing is that sales are still down."

– Dr. Sylvain Charlebois, of the University of Regina, on a study he helped conduct of the Canadian food safety system. The study sought to understand the consumer's perception of food recalls. According to Dr. Charlebois, people are loath to admit they are scared, and the numbers bear that out. Canadian Leader-Post, 4/12/07

Strike at Southern California Red Cross (continued from page 5)

The job action was not expected to threaten the local blood supply. Southern California already imports about 40 percent, and Red Cross officials said it was possible more might be shipped in as a result of the walkout.

Both Red Cross and union officials called for the public to continue donating blood. The union distributed lists of local hospitals where people could give blood, and the Red Cross directed people to the national Red Cross blood donation Web site, www.givelife.org (Sources: Associated Press, 4/30/07; *Los Angeles Times*, 5/3/07) ♦

Israel Changes Blood Donor Deferral Criteria for vCJD, Hepatitis

Israel's national blood service Magen David Adom (MDA) has changed its variant Creutzfeldt-Jakob disease (vCJD) donor deferral criteria to allow anyone who lived in France from 1980 to become a blood donor in Israel. Those who lived in England, Ireland and Portugal for a decade after 1980, when England's bovine spongiform encephalopathy ("mad cow") epidemic began, are still barred from donating blood in Israel and Europe.

MDA blood services director Eilat Shinar, MD told *The Jerusalem Post* last month (4/16/07) that the prevalence of vCJD is around 600 per million in England and between 17 and 20 per million in Ireland and Portugal, but only 1.7 per million in France and thus too small to be a risk factor. For this reason, the European authorities and subsequently MDA (with Health Ministry approval) decided to liberalize the policy for French immigrants and tourists, Dr. Shinar said.

In addition, MDA shortened the deferral period from one year to six months for people who received a blood transfusion, lived with a patient who had hepatitis B or C, had a tattoo done or underwent an endoscopic examination including a biopsy of the stomach or small intestine. (Deferral for endoscopic examinations is based on the fact that the reuse of endoscopes used for biopsy theoretically can transmit viral infections or vCJD if not thoroughly sterilized).

Finally, anyone who was bitten by an unidentified and untested animal now can donate blood in Israel two months after the bite instead of the previous 12 month deferral. ♦

別紙様式第2-1

No. 11

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. 5. 3	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況	Seitz R, von Auer F, Blümel J, Burger R, Buschmann A, Dietz K, Heiden M, Hitzler WE, Klamm H, Kreil T, Kretzschmar H, Nubling M, Offergeld R, Pauli G, Schottstedt V, Volkers P, Zerr I. Biologicals. 2007 Jun;35(2):79-97. Epub 2007 Feb 21.	公表国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)			ドイツ

研究報告の概要

○vCJDが血液供給へ及ぼす影響

変異型クロイツフェルトヤコブ病(vCJD)は、現状では剖検によってのみ確定診断が可能な、不治の神経変性疾患である。現在までの罹患者約200名のほとんどは、感染牛肉が広く食物環に入り込んだ英国在住者である。英国の3症例によってvCJDが輸血により感染する可能性が示された。BSEおよびvCJDは英国以外の複数の国に広がっているため、異なる国及び地域に特異的な評価を実施することが賢明であると思われる。本レビューは、当該リスクの評価及び予防的手段の検討においてドイツで採用された方法を説明するものである。これは、ウシの飼料連鎖およびヒトの食物環から、汚染物質が確実かつ恒久的に除去できるとの基本前提をとるものである。一方でこのモデルは輸血を介したvCJDの伝播が永続化するような可能性があるかどうかという疑問を新たにもたらす。しかし、実際の集団データを基にしたモデル計算は、そのようなことはないであろうことを示唆した。また、輸血経験者を供血者から排除することは、輸血の安全性向上に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。したがって、ドイツでは輸血経験者の排除は推奨されなかった。

報告企業の意見	今後の対応
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く、輸血経験者を供血者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との報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を導入し、平成19年1月16日には全ての輸血用血液への保存前白血球除去の導入が完了した。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

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Biologicals 35 (2007) 79–97
IABS BIOLOGICALS
www.elsevier.com/locate/biologicals

Review

Impact of vCJD on blood supply

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Received 16 November 2006; revised 9 January 2007; accepted 9 January 2007

Abstract

Variant Creutzfeldt–Jakob disease (vCJD) is an at present inevitably lethal neurodegenerative disease which can only be diagnosed definitely post mortem. The majority of the approximately 200 victims to date have resided in the UK where most contaminated beef materials entered the food chain. Three cases in the UK demonstrated that vCJD can be transmitted by blood transfusion. Since BSE and vCJD have spread to several countries outside the UK, it appears advisable that specific risk assessments be carried out in different countries and geographic areas. This review explains the approach adopted by Germany in assessing the risk and considering precautionary measures. A fundamental premise is that the feeding chain of cattle and the food chain have been successfully and permanently cleared from contaminated material. This raises the question of whether transmissions via blood transfusions could have the potential to perpetuate vCJD in mankind. A model calculation based on actual population data showed, however, that this would not be the case. Moreover, an exclusion of transfusion recipients from blood donation would add very little to the safety of blood transfusions, but would have a considerable impact on blood supply. Therefore, an exclusion of transfusion recipients was not recommended in Germany.

Keywords: Bovine spongiform encephalopathy; Variant Creutzfeldt–Jakob disease; Blood supply; Risk assessment

Abbreviations: AFSSAPS, Agence Française de Sécurité Sanitaire des Produits de Santé (French medicinal products authority); BSE, bovine spongiform encephalopathy (degenerative neurological disease in cattle caused by prions); CJD, Creutzfeldt–Jakob disease (TSE disease in humans, transmissible via medicinal products (iatrogenic) or occurring sporadically); FFP, "fresh frozen plasma"(plasma for transfusion); GBR, "geographical BSE risk": classification of countries into one of four risk classes (GBR I–IV) by the Scientific Steering Committee of the European Commission; GSS, Gerstmann-Sträussler-Scheinker syndrome (a human TSE); HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus (agent of AIDS); i.c., intracerebral; IU, infectious unit; i.v., intravenous; M, methionine; PMCA, protein misfolding cyclic amplification (method for amplification of PrP^{Sc} in vitro); PrP, prion protein; PrP^{c}, cellular, physiological form of the prion protein (c = cellular); PrP^{Sc}, pathological form of the prion protein (Sc = Scrapie); RBCC, red blood cell concentrate; SCMPMD, Scientific Committee on Medicinal Products and Medical Devices of the European Commission; SRM, specified risk material (bovine materials in which the BSE agent can be detected in high concentrations (brain, spinal cord etc.)); SSC, Scientific Steering Committee of the European Commission; TSE, transmissible spongiform encephalopathy (disease of the brain, generic term for neurological disorders caused by prions); UK, United Kingdom (Great Britain and Northern Ireland); V, valine; vCJD, variant Creutzfeldt–Jakob disease (human TSE caused by the BSE agent, first described in 1996).

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1045-1056/07/$32.00
doi:10.1016/j.biologicals.2007.01.002

1. Introduction

A working group was formed in 2001 by request of the German Federal Ministry of Health that consisted of staffs from the Paul-Ehrlich-Institut, the Robert-Koch-Institut and the Federal Ministry of Health, as well as external experts. The task of this working group has been to assess the risks for the blood supply in Germany with regard to vCJD and to prepare reports outlining a strategy. The spread of bovine spongiform encephalopathy (BSE) among cattle is believed to be the origin of the problem, followed by the transition to humans via the food chain. Since the epidemical course shows geographical differences, every country needs to assess its specific vCJD risk as a condition for developing a reasonable national blood supply strategy. The group published reports[1] in 2001 and in 2006 [1]. This review summarizes the current view of the group of the impact of vCJD on blood supply.

2. The Occurrence of BSE

Feeding of ruminant material to cattle has most probably caused the occurrence of BSE, a disease of cattle that was first diagnosed in the UK in 1986 [2]. Technological changes (pressure and temperature conditions) in the manufacture of meat and bone meal and other products are considered to be the cause for the occurrence of BSE in the UK beginning in 1985, since the inactivation of the BSE pathogen was no longer sufficiently effective [3]. This assumption is confirmed by the course of the epidemic in the UK where a decline in the number of cases was observed during the mid-1990s with a time lag representing the incubation time of 4–5 years for BSE following the ban on the feeding of meat and bone meal and the regulations on the disposal of BSE-infected animal carcasses [4] (Table 1). While in the first few years it was assumed that there was only one strain of BSE in cattle, several authors have described atypical BSE cases in the past few years [5–7]. These cases do not represent a uniform strain and are characterized by an altered molecular weight of the accumulated PrP^{Sc}, a different anatomical distribution pattern of the pathological changes and the PrP^{Sc} deposits, and partly by the occurrence of amyloid plaques. All cases of atypical BSE described so far have been found in animals older than 8 years. The cases described in France show a biochemical similarity with the cases of scrapie in sheep. Therefore, the possibility that these might be scrapie infections in cattle is discussed.

Through animal trade and trade of feeding stuff components produced from animal carcasses and slaughtering by-products (bone meal, fats for milk replacers, grieves etc.), BSE spread from the UK to other European countries and countries outside Europe (e.g. Canada, Japan, Israel). First Ireland (1989), then Switzerland (1990) and France (1991) reported cases of BSE. During the mid-1990s, Portugal (1994), the Netherlands (1997), Belgium (1997), Luxemburg (1997), and Liechtenstein (1998) reported cases. Toward the end of the 1990s, it became clear that almost all countries with extensive exchange of goods within the European single market during the previous decade were affected by BSE. It was, therefore, not surprising that BSE was diagnosed in some cattle of Denmark, Germany, and Spain in the year 2000 and also in Austria, the Czech Republic, Finland, Greece, Italy, Slovakia, and Slovenia in 2001. Since 2002, BSE has also been diagnosed in Polish cattle. Cases of BSE in cattle imported from the UK were reported as early as the early 1990s by several European countries (Portugal 1990, Germany 1992, Denmark 1992, Italy 1994). Three BSE cases have so far occurred in the United States, of which one animal had been imported from Canada. The two indigenous cases were of the atypical BSE type of which the origin is still unknown.

In addition to animal trade and trade with animal products, however, intrinsic national factors influenced the occurrence and spread of BSE. Since by the 1980s most EU member states had changed their animal carcass disposal methods and processed side products from abattoirs without the removal of risk materials under pressure and temperature conditions that were not sufficient for the inactivation of the BSE pathogen, this pathogen was continuously spread, thus increasing the number of BSE cases. Moreover, only passive monitoring systems based on the reporting of clinical symptoms were in place; BSE rapid tests were not yet available.

Organs and tissues of BSE infected cattle in which the pathogen has been detected are called "specified risk materials" (SRM). SRM of naturally infected animals may, especially toward the end of the incubation period and during the development of clinical BSE symptoms, contain the pathogen in very high concentrations. Using biological detection systems for the BSE pathogen, which include a species barrier, e.g. intracerebral infection into mice, 10^5 infectious units/g SRM (brain) were determined, while a 1000-fold increased infectivity titer is assumed for transmissions within a species [8–10]. The Scientific Steering Committee (SSC) of the European Commission set up an SRM list for cattle (e.g. skull including brain and eyes, tonsils, spinal cord) (SSC 1998[2]), which served as a basis for various European policies for the exclusion of SRM in the food and feed chains. Since the spread of the BSE crisis in Europe, the definition of specified risk materials has been revised several times (a comprehensive overview of the European legislation can be found in Table 2 of [1]). According to the latest amendment, the tissues designated as SRM must be subjected to safe removal and must not enter the food chain. The following tissues are designated as SRM: "The skull excluding the mandible and including the brain and eyes, the vertebral column excluding the vertebrae of the tail, the spinous and transverse processes of the cervical, thoracic and lumbar vertebrae and the wings of the sacrum, but including the dorsal root ganglia, and the spinal cord of

[1] The reports published by this group in German language in the years 2001 and 2006 can be found in the internet: http://www.pei.de.

[2] Scientific Steering Committee (SSC), 1998. Listing of Specified Risk Materials: a scheme for assessing relative risks to man—Opinion of the SSC adopted on 9 December 1997 (Re-edited version adopted by the SSC during its Third Plenary Session of 22–23 January 1998).

Table 1
Number of BSE cases reported

Country	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005[a]
Austria	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
Belgium	0	0	0	0	0	0	0	0	1	6	3	9	46	38	15	11	1
Canada	0	0	0	0	1*	0	0	0	0	0	0	0	0	0	2	1	1
Czech Republic	0	0	0	0	0	0	0	0	0	0	0	0	2	2	4	7	8
Denmark	0	0	0	1*	0	0	0	0	0	0	0	1	6	3	2	1	n.d.
Finland	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	n.d.
France	0	0	5	0	1	4	3	12	6	18	31	161	274	239	137	54	n.d.
Germany	0	0	0	1*	0	3*	0	0	2*	0	0	7	125	106	54	65	32
Greece	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	n.d.
Ireland	15	14	17	18	16	19	16	73	80	83	91	149	246	333	183	126	69
Israel	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Italy	0	0	0	0	0	2*	0	0	0	0	0	0	48	38	29	7	3
Japan	0	0	0	0	0	0	0	0	0	0	0	0	3	2	4	5	7
Liechtenstein	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	n.d.
Luxembourg	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1
The Netherlands	0	0	0	0	0	0	0	0	2	2	2	2	20	24	19	6	n.d.
Poland	0	0	0	0	0	0	0	0	0	0	0	0	0	4	5	11	18
Portugal	0	1*	1*	1*	3*	12	15	31	30	127	159	149	110	86	133	92	37
Slovakia	0	0	0	0	0	0	0	0	0	0	0	0	5	6	2	7	n.d.
Slovenia	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	1
Spain	0	0	0	0	0	0	0	0	0	0	0	2	82	127	167	137	75
USA	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Switzerland	0	2	8	15	29	64	68	45	38	14	50	33	42	24	21	3	3
United Kingdom	7228	14407	25359	37280	35090	24438	14562	8149	4393	3235	2301	1443	1202	1144	611	343	151

Source and information on up-to-date statistics: Office International des Epizooties, as of 9 January 2006 (www.oie.int). *Cases in imported animals.
[a] Data for 2005 still incomplete. n.d., not done.

bovines aged over 12 months, and the tonsils, the intestines from the duodenum to the rectum and the mesentery of bovines of all ages; the skull including the brain and eyes, the tonsils and the spinal cord of ovine and caprine animals aged over 12 months or which have a permanent incisor erupted through the gum, and the spleen of ovine and caprine animals of all ages." Because of the significant decrease in the number of BSE cases in the European Union, the age limit for the collection and safe removal of SRM for the spinal cord of bovine animals was raised to 24 months and a raise of the test age is being discussed.[3]

The SSC has developed a procedure by which the geographical BSE risk (GBR) in a member state or non-European country can be evaluated. In its opinion, published in July 2000,[4] it laid down the following criteria for classifying one of four risk levels:

- Structure and dynamics of the bovine population,
- BSE surveillance,
- Cullings in connection with BSE cases,
- Imports of bovine animals and meat and bone meal (MBM),
- Feeding,
- Ban on the feeding of meat and bone meal (MBM bans),
- Regulations concerning specified risk material (SRM bans),
- Removal of animal carcasses.

The risk levels are defined in Table 2.

At that point in time (2000), Argentina, Australia, Chile, Norway, New Zealand, and Paraguay were classified as GBR level I, Austria, Finland, Sweden, Canada, and the United States as GBR level II, whereas the UK and Portugal were classified as GBR level IV. All other countries, including Germany, were classified as GBR level III. Germany's classification as GBR level III caused heated discussions in Germany, since up to that time the country had been considered to be absolutely BSE free. In actuality, all countries rated into BSE level III indeed identified BSE cases in their own countries within the following months.

Since 2001, the GBR has been assessed for various other countries, e.g. candidate countries for accession to the EU. Almost all countries were classified as GBR level III, since insufficient monitoring had been carried out to guarantee satisfactory statistical safety. A number of countries evaluated in 2000 were later re-evaluated, which led to the classification of Austria, Canada, USA, Mexico and South Africa to GBR level III. In March 2003, Canada's second BSE case was discovered (the first case was diagnosed in 1993), and in June 2005, the first BSE case was confirmed in the USA.[5]

[3] See the "BSE road map" for more details, http://europa.eu.int/comm/food/food/biosafety/bse/roadmap_en.pdf

[4] Final Opinion of the Scientific Steering Committee on the Geographical Risk of Bovine Spongiform Encephalopathy (GBR). Adopted on 6 July 2000.

[5] The results and opinions of the Scientific Steering Committee (SSC) and the European Food Safety Authority (EFSA) can be found in http://europa.eu.int/comm/food/fs/sc/ssc/outcome_en.html and http://www.efsa.eu.int respectively.

Table 2
GBR levels as defined by the SSC

GBR level	Presence of one or more cattle clinically or pre-clinically infected with the BSE agent in a geographical region/country
I	Highly unlikely
II	Unlikely but not excluded
III	Likely but not confirmed or confirmed, at a lower level
IV	Confirmed, at a higher level

The member states and third countries were also classified into five BSE status categories.[6] The classification in status categories was based on criteria similar to those of the SSC. However, in this context the number of diagnosed BSE cases served as an important additional factor. Consequently, other points of combating BSE laid down in this EU regulation refer to the status category of the appropriate country, such as the required extent of the safe retrieval and removal of SRM.

Following the steady decrease of BSE cases in the UK in the past few years, the number of BSE cases reported per one million bovine animals older than 30 months has fallen below 1,000, enabling a re-evaluation of the UK. The application was given a favorable opinion by the European Food Safety Authority (EFSA), and it was suggested that the UK be classified as BSE risk status III. A change in the BSE risk status represents a significant relief for the UK regarding international trade of bovines and bovine animal products.

3. BSE in Germany

Passive BSE surveillance has been performed in Germany for years, i.e. all bovine animals that died or became clinically sick due to disorders of the central nervous system and were suspected to have suffered from BSE were examined. The brains of such animals were subjected to histopathological examination, and any samples with abnormal results were also examined for plaques of PrP^{Sc} by immunohistochemical examination and/or scrapie associated fibril (SAF) extraction with subsequent immunoblot. These examinations did not reveal any BSE cases in German cattle.

The first BSE rapid test, the Prionics Check Western blot developed by Prionics (Switzerland), became available in mid-1999. Even though the test had not yet been approved, it was already used in some European countries. A series of examinations using this BSE test for 5,000 beef cattle was carried out between March and May 1999 in North Rhine-Westphalia, Germany. All these animals showed negative results, reinforcing the hope of a BSE-free Germany.

In preparation for transposing the Commission Decision 2000/374/EC, which established random BSE monitoring of bovine animals, a few voluntary BSE examinations were carried out in cattle samples starting in mid-November of 2000. These examinations revealed the first indigenous German BSE case in Schleswig-Holstein confirmed by the National Reference Laboratory on 26 November 2000. This was followed by the introduction of rapid test examinations throughout Germany within a short period of time. After the extensive introduction of BSE rapid tests in December 2000 for all slaughtered cattle as well as for fallen stock (first over 30, then over 24 months old; since June 2006 again over 30 months old), 390 BSE cases were identified in following years (reference date: 16 January 2006) (Table 1). The number of cases reported annually is steadily declining, despite a slight increase from 2003 to 2004.

Altogether, these data indicate that the BSE "epidemic" in Germany may have already exceeded its peak before the first case was even diagnosed. Simultaneous to the introduction of the BSE rapid test, a total ban on feeding protein-containing products and fats derived from warm-blooded land animals to ruminants throughout Europe was imposed in the year 2000. In Germany, this ban was extended to the feeding of all productive livestock as defined in the Futtermittelgesetz (Act on Feeding-Stuffs).

While during the first two years of BSE monitoring in Germany the disease was predominantly diagnosed in animals born in 1995 and 1996, BSE has been increasingly identified in animals born in later years (particularly in 1998/99) since 2004. This suggests that after a significant entry of BSE infectivity into the feeding-stuff chain in 1995/96, a reduction must have occurred, followed by a second increase in the pathogen content around 1998/99. It is still unknown what caused these two BSE waves. Until the end of 2004, BSE was diagnosed in ten bovine animals born in 2000. Then, in April 2005, BSE was diagnosed for the first time in a bovine born in May 2001, i.e. after the implementation of the total feed ban of MBM from warm-blooded land animals to productive livestock in Germany. A second case followed in June 2005 when a BSE infection was diagnosed in an animal born in March 2001. It must be assumed that these two cases were caused by a contamination with the pathogen beyond the feed ban. In this context, it must be mentioned that in the UK, 95 cases born after the reinforced feed ban of August 1996 (so-called BARB-BSE cases) were diagnosed up to April 2005 (source: DEFRA-statistics). Two explanations must be considered as the cause for the occurrence of such cases:

1. The routes of infection have not yet been fully identified, and a transmission cannot be excluded 100% despite a strict adherence to the feed ban.
2. In isolated cases, MBM was fed to animals even after the feed ban came into force. This is very difficult to prove after so many years, and would imply that the control mechanisms might have to be made even more restrictive.

4. BSE in small ruminants

The theoretical risk of transmission of the BSE pathogen to small ruminants has been scientifically discussed for some

[6] Regulation (EC) No 999/2001 of the European Parliament and the Council of 22 May 2001 (Official Journal of the European Communities of 31 May 2001, L147, p. 1) laying down rules on prevention, control and eradication of certain transmissible spongiform encephalopathies.

time, resulting in the introduction of an active TSE surveillance of these species by rapid BSE test pursuant to Regulation (EC) 999/2001. After the introduction of this intensive monitoring, the number of reported TSE cases in small ruminants markedly increased in nearly all member states.

In Germany, 0–3 cases of scrapie had been diagnosed for many years; the figure has risen to 31–119 individual animals with altogether 68 outbreaks of classical and atypical scrapie per year since 2002. In some cases, the disease could be detected in up to 56 animals of the same herd. In Germany, no TSE infection has been diagnosed thus far in any of the 12,000 goats tested since the beginning of the intensified monitoring. Regulation (EC) 999/2001 also laid down that each TSE case in small ruminants was to be tested by means of biochemical methods or animal experiments ("strain typing"). This measure serves to guarantee that a possible BSE infection in these small ruminant species would not remain undetected. Since the animal experimental methods used up to now mainly for scientific interest [11,12] are very time-consuming and costly, the samples are usually first tested by means of biochemical methods (analysis of the molecular weight, the glycosylation profile, and the antibody binding affinity of the accumulated pathological prion protein) [13–17]. So far, evidence of BSE infection in sheep has not been found in any of the 37 classical scrapie outbreaks in Germany [17], nor during the relevant tests performed in other member states. The cases of atypical scrapie were excluded from the strain typing, since this TSE type is clearly distinct from BSE [18]. Active surveillance in France, however, and subsequent strain typing tests gave clear evidence of a BSE infection in a goat that had been diagnosed with a TSE infection in 2002.

This first evidence that the BSE pathogen can cross the species barrier between cattle and small ruminants gave rise to special concern in expert circles; in small ruminants, the TSE pathogenesis clearly differs from that in cattle. In cattle, pathological prion protein and BSE infectivity remain strictly limited to the central nervous system and only become detectable immediately before the occurrence of clinical symptoms [19]. The combination of rapid testing of all beef cattle above a certain age (30 months in the EU, first 24 months which was raised to 30 months in June 2006 in Germany), in combination with the safe removal of SRM, thus presents an effective consumer protection measure. The situation is different in sheep, where the pathogen can be detected in various organ systems very soon after the infection, above all in the nervous and lymphatic systems [20–22]. It is therefore well possible that an animal testing negative in the rapid test of the brain stem has already accumulated disease-related prion protein and infectivity in other organs. Since, however, TSE pathogenesis in sheep depends on various factors, e.g. the PrP^{Sc} genotype of the affected animal and the TSE strain, no uniform testing concept for this animal species would guarantee TSE detection at the earliest possible point in time after infection. Any BSE infection in small ruminants thus presents a potentially enhanced risk for the consumer as compared to the occurrence of the same disease in cattle.

Table 3
Patients with vCJD worldwide and duration of stay in the UK

Country	Total number of cases (number alive)	Cases with cumulative residence in UK > 6 months during the period 1980–1996
UK	162 (6)[a]	162
France	20 (3)	1[b]
Republic of Ireland	4 (1)	2
The Netherlands	2 (1)	0
USA	2 (0)	2
Canada	1 (0)	1
Italy	1 (0)	0
Japan	1[c] (0)	0
Portugal	1 (1)	0
Saudi Arabia	1 (1)	0
Spain	1 (0)	0

[a] As of 7 August 2006 (http://www.cjd.ed.ac.uk).
[b] The person from France had traveled regularly to UK over more than 10 years since 1987.
[c] The person from Japan had resided in the UK for 24 days in the period 1980–1996.

5. The occurrence of vCJD

First described in the UK in 1996, vCJD can be distinguished from the classical forms of CJD both by its clinical and neuropathological characteristics [23–25]. The numbers of cases observed world-wide are shown in Table 3.

Out of the 162 confirmed or probable cases diagnosed in the UK, 156 patients have died, and in 112 cases the diagnosis was neuropathologically confirmed. One Chinese patient who died in Hong Kong had stayed in the UK for several years and is included in the UK cases. The number of vCJD deaths in the UK reached its peak in 2000 with 28 cases; then, the number of deaths due to vCJD dropped sharply through 2005. This development currently supports the hope that the epidemic has surpassed its peak in the UK. This assumption, however, is still unsafe due to the lack of knowledge about the disease, duration of the incubation period, and frequency of manifestation dependent on the genotype at codon 129 of the prion protein gene.

As of 28 July 2006, 20 cases have been diagnosed in France. The number of probable and confirmed vCJD cases in France has not shown any decline; 3 cases were diagnosed in 2004 and 6 cases in 2005.[7] Seventeen individuals in France have died of vCJD. The number of persons who died of probable or confirmed vCJD in the UK and in France up to 2005 is shown in Fig. 1 (status of the data UK: 3 March 2006, France: 28 February 2006).

It is assumed that vCJD is caused by the same pathogen as BSE in cattle. This is based on the geographic occurrence of BSE and vCJD, the biochemical similarity between BSE and vCJD associated prion proteins [26,27], the non-distinguishability of the pathogen strain typing (incubation periods in different mouse strains, lesion patterns in the brain) [28,29], induction of neuropathological changes in macaques after

[7] http://www.invs.sante.fr/surveillance

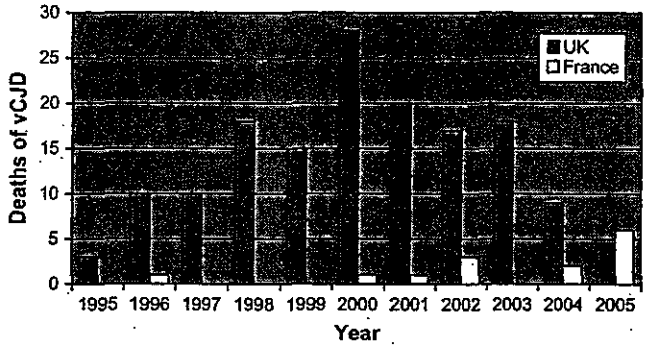

Fig. 1. Deaths caused by probable and confirmed vCJD in the UK and France from 1995 to 2005.

infection with BSE material very similar to those in vCJD patients [30], identical biological characteristics during transmission of BSE and vCJD material to transgenic mice [31], and comparable pathogen characteristics (e.g. lesion patterns, PrP^{Sc} protein patterns) [32].

It is assumed that infection of humans occurs via the food chain by contaminated beef. It is highly likely that for food production, tissue of infected animals with high pathogen content, especially brain and/or spinal cord tissue, was used consciously or unconsciously. The route of infection from uptake of the pathogen in the gastro-intestinal duct via the N. vagus and the N. splanchnicus into the central nervous system could be shown experimentally [33]. A possible transmission of pathological prion by medicinal products, medical devices, and cosmetics containing bovine material seems to play at best a subordinate role, since the analysis of the vCJD cases up to now for possible risk factors did not reveal any suspicion.[8]

Concerning human to human transmission, there is currently no evidence for transmission of vCJD by transplants or other medicinal products derived from human material, e.g. plasma derivatives, albeit transmission by this route cannot be excluded in principle. However, three vCJD cases are seriously suspected to have been caused by blood transfusions (cf. Section 7).

Apart from this, there are no reports on iatrogenic vCJD infections worldwide, contrary to classical CJD, which was transmitted in more than 100 cases by pituitary (growth hormone, follicle stimulating hormone) and dura mater products. In isolated cases, infection by corneal transplantation and by reused surgical instruments (intracerebral electrodes) was reported [34]. The transmission risk was minimized by suitable measures (e.g. replacement of the pituitary extracts by recombinant products, critical selection of dura mater and cornea donors, treatment of the dura mater with sodium hydroxide solution, use of disposable instruments).

In contrast to the classical forms of CJD, however, vCJD patients have measurable pathogen content not only in the central nervous system but also in peripheral tissues [35], especially in lymphatic tissues (tonsil, appendix, spleen). It is therefore conceivable that infection is possible in principle by reuse of instruments in general surgery, including flexible endoscopes. Recommendations for minimizing iatrogenic vCJD transmissions were put forth in April 2002 [36], with notes on the validation of decontamination [37], and the testing of new, instrument-compatible methods [38].

6. Estimation of the extent of the spread of vCJD

Mathematical models have been developed to assess the extent of the vCJD epidemic. Estimates would require sufficiently reliable information on relevant parameters, e.g. minimum infectious dose in the event of oral route of transmission, extent of consumption of contaminated beef, distribution of the incubation periods, and information on the susceptibility of the exposed population. These basic parameters are still not sufficiently known, and every model calculation is therefore inevitably fraught with uncertainties. As a rule, the models take into account the uptake of the pathogen via contaminated beef only. Since human to human transmission might add to the epidemic, infectivity of parenteral administration of the pathogen needs to be known. No estimates have so far been published on the portion of possible vCJD cases that follows this infection route.

Models developed to assess the vCJD epidemic in the UK initially assumed that only a portion of the population can contract the disease, based on the observation that clinical vCJD has developed only in individuals who are homozygous for methionine (M/M) at codon 129 of the prion protein gene. This applies to approx. 40% of the Caucasian population (Table 4) [39–43].

In each model, different incubation periods for vCJD (up to 60 years) and differing age-related susceptibilities were taken into account. The models improved with the increase of data on the actual progression of the epidemic. The estimated number of future vCJD cases in the UK caused by food of up to several million [44] could be revised first to 136,000 [45] and later to 7,000 [46].

Currently, the incubation period and degree of susceptibility of the exposed population are still uncertain. Above all, the polymorphism at codon 129 seems to play a role in individual susceptibility. While up to now all vCJD patients were homozygous M/M, in the year 2004 a transfusion recipient who was heterozygous at codon 129 (methionine/valine, M/V) was

Table 4
Polymorphism of the prion protein gene in the general population, in CJD and vCJD patients

Individuals tested	M/M	M/V	V/V
General population	39–48%	42–50%	10–13%
Sporadic CJD	69–78%	12–15%	10–16%
vCJD[a]	100%	0%	0%

M: methionine, V: valine.
[a] Clinical vCJD cases.

[8] The National Creutzfeldt–Jakob Disease Surveillance Unit (UK): www.cjd.ed.ac.uk

diagnosed with pathological prion in the lymphatic tissue. He died of rupturing aortic aneurysm 5 years after the transfusion without any evidence of a neurodegenerative disorder. This case suggests that individuals not homozygous for methionine at codon 129 can be infected. It remains unclear whether those heterozygous individuals would stay as a potential asymptomatic vCJD carrier, or develop the disease only with delay.

The existence of a possible "carrier status" is supported by a retrospective serial analysis of appendix and tonsil material in the UK, where 3 of 12,674 appendices tested revealed pathological prion [47,48]. Recently, the genotype of two of these individuals who had not developed vCJD at the time of the examination was sequenced (the third sample was not available for analysis) and found to be homozygous for valine at codon 129 of the prion protein gene [49]. Immunohistochemical examinations revealed a different prion distribution in two out of the three cases from that found in the lymphatic tissue of vCJD patients. It is currently unclear whether this might be indicative of the outcome of the disease. Methodological problems in the evaluation with regard to sensitivity and specificity might play a role. In addition, it must be borne in mind that the studied patients were not representative of the general population due to age distribution. If this random sample of histological examinations is used as a basis, and 100% sensitivity and specificity of the test used is assumed, the estimated prevalence of undetected vCJD infections per 1 million inhabitants in the UK would amount to 235 (49–692). This would mean a higher prevalence of vCJD than previously estimated on the basis of the decreasing figures of clinical cases.

The mathematical models were adapted to these new findings; wider genetic susceptibility and a possible carrier status were assumed for the disease. Taking into account the remaining uncertainties on the length of the incubation period, the estimated number of clinical vCJD cases by the year 2080 is 70 (10–190) based on the existing calculation model and a model for a carrier status [50], as opposed to 363 cases (no confidence interval indicated), based on a more pessimistic assumption. If the data on the examinations of the appendices [48] are taken into account, the estimation is 133 (32–3,780) cases [50].

The model published by Clarke and Ghani in 2005 provides estimates for the number of individuals with subclinical and preclinical infection with the vCJD pathogen [50]. The histological data of the appendices were taken into account in this assessment, and 50% sensitivity of the tests for subclinical infection was assumed. Based on these assumptions, a far greater number of individuals infected but without clinical manifestation (1,130–13,440) can be assumed. The number of these carriers and the question of whether they would be infective are important for possible iatrogenic transmission and may markedly influence the absolute number of future vCJD cases. Besides transmission by blood products, incomplete disinfection of surgical instruments might also play a part.

The above model calculations refer to the UK. For countries without or with only a small number of vCJD cases, the estimate is even more uncertain. The decisive parameter is the extent of exposure to food stuffs produced from infected beef. A synopsis of the peak incidence of the BSE epidemic in various countries, as well as the assumed period of exposure to BSE (2001 report, endnote 1) clearly show that the extent of the BSE epidemic in the UK is a multiple of that of other countries, even if differences in the reporting criteria are taken into account. A risk of exposure for countries with no or only few BSE cases can only be estimated by the extent of imports of beef cattle from the UK within the relevant period of time. Fig. 2 shows imports of beef from the UK between 1990 and 1995.

The mathematical models from the UK on estimating the vCJD epidemic were used in Ireland and France, taking into account the actual situation in these countries. In Ireland, where four cases of vCJD have occurred up to now (two of them were residents of the UK for a considerable period of time), an estimation was performed on the basis of the model developed in the UK with adaptations for conditions in Ireland [51]. The estimation considers potentially contaminated Irish cattle, cattle imports from the UK, and the consumption of British beef during visits to the UK. This model, too, takes into account only the group of individuals who are homozygous for methionine at codon 129. It was estimated that 1–2 (0–46) more clinical cases of vCJD would occur in Ireland. Apart from the above limitations, the adapted model is suitable for performing estimation for countries with few or no cases of vCJD if the basic data are known. In France, 20 vCJD cases have been reported so far. In a current model calculation, also based on the epidemiological data from the UK, it was estimated that after 2004, another 33 vCJD cases (12 of them in 2004 and 2005) would occur [52]. The model calculation takes into account imports of British beef to France, beef consumption and travel to the UK. The estimate of the case numbers for France has decreased by two thirds compared with the previous forecasts from 2000 [53].

No case of vCJD has so far been diagnosed in Germany. Since the epidemiological situation in Germany is hence markedly different from that in the UK, and, in addition, the extent of exposure to potentially BSE contaminated beef cannot be accurately quantified, no primary data are available, allowing a valid use of models for estimating the incidence of primary vCJD cases in Germany. Based on estimates for France and Ireland, where only a few vCJD cases have been diagnosed, it can be assumed on the basis of the current state

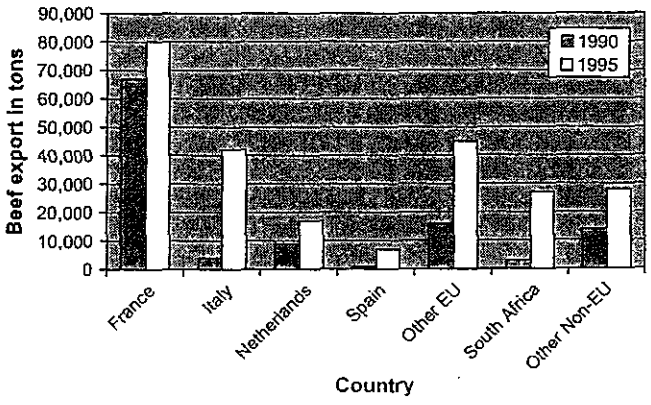

Fig. 2. Beef imports from the UK in tons.

of knowledge that only isolated cases of vCJD will occur in Germany.

7. Risk of vCJD transmission through blood (secondary infections)

Various approaches, such as animal experiments and epidemiological and case control studies, as well as the observation of individual cases, address the question of whether blood and blood products constitute a risk of vCJD transmission.

A number of experiments explore the possibility of prion transmission by blood and its components, with great variety of combinations of TSE agents and animal species [42,54,55]. Many results, however, must be interpreted with a number of restrictions. The investigational material of the donor animals (blood, serum, cells etc.) was usually given to the indicator animals by i.c. administration. This permits a more sensitive detection of the pathogen, but conclusions regarding i.v. administration are difficult. Secondly, many experiments were performed with animals infected in a "non-natural" manner, which makes the extrapolation of the corresponding results more difficult. In addition, the tests often involve a species barrier, which means a decrease in sensitivity.

Despite many contradictory results, the following can be concluded from small rodent experiments:

- In principle, infectivity can be detected in the blood of experimentally infected animals.
- Titers of infectious TSE agent in the blood of artificially infected animals were found to be very low (1–100 infectious units/ml) in sensitive detection systems. The question arises in some experiments as to what extent the detected infectivity really reflects replicated agent and not just residual inoculum.
- While in various experiments i.v. application has led more rarely to infection of the indicator animal than i.c. inoculation, in a recent experiment using mice infected with either mouse-adapted strains of GSS, a special familial form of CJD, or vCJD, no major difference was found between i.v. and i.c. inoculation. Infectivity of approx. 20–30 IU/ml was found in the preclinical and the symptomatic phases 17 and 23 weeks after inoculation in both the buffy coat of the blood and in the plasma [56].

A transfusion experiment, where blood for transfusion was drawn from sheep in two series of experiments, pointed to a potential transmissibility of prion diseases by blood [57]. The donor sheep had been either orally infected by brain material from BSE infected cows or were sheep with particular genetic susceptibility for scrapie. After transfusion of whole blood or buffy coat of those donor animals that later died of the orally induced "BSE" or scrapie, definite TSE developed in a number of recipient animals. The identification of the pathogen confirmed transmission in this experiment. It must be noted that the recipient animals were genetically sensitive to TSE.

Formal retrospective epidemiologic studies and case control studies did not reveal any evidence of transmission of human TSEs by blood or blood products. In contrast to a number of viruses (HIV, HCV, HBV), no suspected case of transmission of classical CJD to a hemophilia patient has become known [58]. Since this disease can barely be overlooked in this well monitored group of patients, this is reassuring.

However, three case reports published in the UK demonstrate the possibility of vCJD transmission by transfusions. No available diagnostic system could prove or exclude vCJD transmission in any individual case. Joint consideration of the published cases, however, does not permit any other conclusion than transmissibility of the vCJD agent by blood transfusion. A monitoring system, the National CJD Surveillance Unit, was established in the UK in 1990, which, among other things, was designed to identify blood donors among vCJD patients and locate their donations. The recipients of these donations were observed, and in the event of their death appropriate tests including an autopsy with histopathological identification of the vCJD agent were performed. 15 of the vCJD patients who were diagnosed up to December 2003 had donated blood. They had donated a total of 55 labile blood products of which 48 had been transfused. At that time 17 living recipients were identified and monitored. This surveillance system has so far identified three cases: In 1996, a then 62-year-old patient received a total of 5 RBCC within one operation. One of these concentrates (non-leukocyte reduced) originated from a donation of a 24-year old individual who was healthy at the time of donation but died of confirmed vCJD in 2000 (3.5 years after the donation). The recipient developed symptoms 6.5 years after the transfusion and died of vCJD 13 months later; the diagnosis was confirmed by a post mortem [59]. Since the recipient lived in the UK, thus also exposed to a risk for contraction of vCJD via the food chain, transmission by transfusion could not be proven but was highly likely considering the low statistical probability of coincidence, i.e. an infection via the food chain not related to the transfusion, rated as 1:15,000–1:30,000. The second case was another elderly patient who received a non-leukocyte reduced RBCC from a donation of an individual who developed symptoms 18 months later and died of confirmed vCJD in 2001 [60]. The recipient died of a non-related cause (rupturing aortic aneurysm) 5 years after the transfusion without any signs of a neurologic-psychiatric disorder. Within the above-described surveillance, a post mortem was carried out. The vCJD agent was found by histopathological examination in the spleen and neck lymph nodes. The agent was obviously transmitted; however, an involvement of the CNS was not detectable—neither clinically nor histopathologically. In contrast to all previously identified vCJD patients, this patient was heterozygous M/V at codon 129. The infection was subclinical at the time of death; whether vCJD would have developed in this case must remain open. A third patient in the UK was reported in 2006 who developed symptoms of vCJD after receiving non-leukocyte depleted RBCC 7 years and 10 months before. The patient is a M/M homozygote at codon 129. The donor of the concentrate developed vCJD 21 months after the donation

[61,62]. A fourth probable case was announced in January 2007 (http://www.hpa.org.uk/hpa/news/articles/press_releases/2007/070118_vCJD.htm).

As long as a blood test for vCJD does not exist, assessment of cases of suspected transmission would be possible only to a limited extent; this applies either if the individual affected by vCJD donated blood or had received transfusions of blood components. Recommendation 33 of the AK Blut (National Advisory Committee 'Blood'), 2006, provides guidance in this context [63]. A detailed statement regarding the safety of blood products in view of vCJD has recently been published by the Scientific Committee on Emerging and Newly Identified Health Risks of the EU Commission, SCENIHR [64].

Of crucial importance for the vCJD risk from transfusion is the number of individuals within a given population who are infected and may carry the agent in their blood. Histological evidence of prions in appendices [48] indicates a higher frequency of infection than previously estimated based on the occurrence of vCJD. As described above, one would now hypothesize that all individuals are susceptible to the vCJD agent, not only those homozygous for M/M, who represent 40% of the population. In another human TSE, Kuru, cases with extremely long incubation periods of up to 56 years have been reported, and all whose PrP gene could be analyzed were not M/M homozygotes [65]. Thus a higher number (maximally double) of infected individuals should be assumed than up to now. If this was conceivable for the British population, we would have to expect one subclinical case in roughly 4,000 people.

8. Reduction of TSE in the manufacture of blood products

In view of the limited knowledge, in assessing the effectiveness of methods the following partly speculative and pessimistic assumptions and remarks of reservation have to be made:

- The amount of infectivity in blood is estimated on the basis of data from animal experiments. The French authority AFSSAPS [66] had previously assumed as worst case scenario that infectivity in whole blood is 100 IU i.c./ml (infectious units/ml in case of intracerebral administration), and lower for intravenous inoculation at 10 IU i.v./ml (infectious units/ml in the case of intravenous administration). However, in primates survival rates after i.v. and i.c. inoculation were similar [29,67]. In addition, in recent comprehensive studies, 13.6 IU i.c./ml were measured in the blood of scrapie infected hamsters [68], and approx. 20 IU i.c./ml in the plasma of mice infected with adapted vCJD or GSS pathogens [56,69]. AFSSAPS now assumes an infectivity of 20 IU i.v./ml in the blood based on this new data. For leukocyte depleted plasma, a pathogen reduction by 50% is assumed, thus 10 IU i.v./ml in leukocyte depleted plasma instead of previously 1 IU i.v./ml plasma [66]. A study performed for the British Health Ministry (DNV-Consulting, 2003) [70] assumes a pathogen content of 10 IU i.c./ml in the plasma and a 5-fold reduced infectivity in the case of i.v. inoculation, thus 2 IU i.v./ml.
- This is extrapolated to vCJD cases even though no infectivity has been found in their blood so far [71,72].
- There are no accurate data during which times infectivity could be present in the blood of individuals during the incubation period and the course of disease.
- The form of infectious prions (association with cells, monomers, multimers, aggregate, fibrils) in the blood of "naturally" infected creatures is unknown. Based on animal experiments [73], it had been assumed that 90% of the infectivity of whole blood would be present in the cellular fraction and 10% in the plasma. More recent studies [56], however, point to an approx. equal distribution of the amount of pathogen in the plasma and in the leukocyte fraction.

9. Blood components for transfusion, leukocyte depletion

Leukocyte depletion (LD) became compulsory (among other reasons) as a precautionary measure against a possible transmission of vCJD by blood components in various countries, including Germany. Treatment of whole blood (2.5×10^9 leukocytes/ml) results in a reduction of leukocytes by 3–4 log steps with residual numbers limited to 10^6 leukocytes per blood component. An experimental study has been conducted into the capacity of LD to remove the TSE pathogen using 500 ml blood of scrapie infected hamsters [74]. The concentration was reduced from 13.1 IU_{50}/ml in whole blood to 7.6 IU_{50}/ml, i.e. 42% of the pathogen were removed during leukocyte depletion. Since the actual pathogen concentration in human blood is unknown, it is difficult to assess to what extent this reduction would represent a gain in safety. No LD of RBCC was carried out in either of the three transmission cases. However, no conclusion may be drawn that such treatment of the components would have prevented transmission.

9.1. Red blood cell concentrates (RBCC)

In Germany, RBCC predominantly originate from whole blood donations. Before LD was enforced (October 2001), buffy coat-free RBCC were the standard preparations which, in an average volume of 250 ml, may contain up to 1.2×10^9 leukocytes, according to the applicable national guideline [75] and the Council of Europe Recommendations. Even after the LD has become mandatory, a potential transmission risk of RBCC must be assumed.

9.2. Platelet concentrates (PC)

Eighty percent of the PC in Germany is manufactured from whole blood donations (WB-PC, e.g. buffy coat, usually pooled from 4–6 donations) and approx. 20% from apheresis (A-PC). Titers of approx. 10 IU/ml [56] were measured in the thrombocyte fraction of mice. Residual infectivity must be assumed even after 42% pathogen reduction by LD of the

whole blood. A preference for A-PC is not justified at present. Assessment of a residual infectivity is difficult since in apheresis high blood volume is processed and the behavior of vCJD infectivity in the apheresis system is difficult to predict.

9.3. Plasma for transfusion ("Fresh Frozen Plasma, FFP")

In Germany, quarantined plasma (Q-P) and solvent/detergent treated plasma (SD-P) are currently available. The market share of SD-P is approx. 10%. SD-P is manufactured by pooling approx. 700–1,200 individual donations. The volume for a unit of Q-P in Germany is approx. 230–280 ml, and for SD-P it is 200 ml.

In a previous assessment (2001 report, footnote 1), the content in cell free plasma had been estimated to be 1 IU i.v./ml; 250 ml of quarantined plasma would contain 250 IU i.v. cell free plasma. Two calculations had been made for SD-P:

(a) Based on the assumption that infectivity is distributed homogenously in the pool, 200 ml individual plasma containing approx. 200 IU i.v. (residual cells neglected, see above) would enter a pool; assuming a low number of 500 donations this would result in the dilution to 0.4 IU i.v. per plasma bag in the SD-P separated after treatment.
(b) Based on the assumption that infectivity is in principle not evenly distributed in portions <1 IU i.v., an infectious donation containing 200 IU i.v. could be distributed to a maximum of 200 plasma bags, i.e. 200 of 500 SD-P would be infectious. Assuming 1 out of 120,000 donations were infectious (AFSSAPS, 2000) and a pool size of 500 donations, the risk would be 1 out of 240 SD-P batches. The risk of an infectious SD-P would thus be approx. 1 in 600 (240 times 2.5), which would be less favorable compared with 1:120,000 for Q-P from an individual donation.

Assuming 10 IU i.v./ml instead of previously 1 IU i.v./ml in the contaminated plasma donation [66], the risk becomes higher to the disadvantage of the pooled plasma. Based on this assumption, the above calculation (a) for a pool of 500 donations and 2,000 IU in a donation would result in an average burden of 4 IU in all plasma bags of a batch. If it was assumed that infectivity in principle is not distributed in units <1 IU i.v. (b), an infectious donation would contain 2,000 IU i.v. in 500 donations so that all 500 plasma bags from a pool of SD-P could be infectious. However, since these calculations contain many unknowns (e.g. reduction effects) and are based on unproven hypotheses, no recommendations are given here as to the preferred type of plasma.

Another question is whether infectivity in the plasma can be reduced by further measures. It has been considered to prepare plasma cell free to the greatest possible extent and to remove cell fragments by filtration through a membrane with appropriately small pores, an approach pursued in France. No experimental evidence is available on whether this could effectively reduce the infectivity of plasma. Furthermore, it is not clear whether the quality of the plasma (e.g. activation of coagulation factors, neoantigen formation) might be impaired. Therefore, a decision in favor of introducing such membrane filtration seems currently premature.

10. Industrial products from pool plasma, nanofiltration

The evaluation of individual fractionation and inactivation steps in the manufacture of plasma derivatives (e.g. factor concentrates, immunoglobulins, albumin), regarding vCJD pathogens and the risk for the recipient is still fraught with uncertainties:

- Some assessments are based on the assumption that existing vCJD infectivity can be pushed below a presumably safe threshold dose by means of several dilution and reduction steps. It has not yet been determined whether an infectious threshold dose administered once would cause infection of the recipient, and whether several doses "below the threshold" would have a cumulative effect.
- Opinions are divided as to whether the size of the fractionation pool plays an important part (analogous with the SD-P):
  - Using a large pool, in case of possible contamination of the products a large number of recipients could be at risk. This would suggest that small pools would have to be used.
  - On the other hand, a freely distributed infectivity (e.g. if prion monomers were present) would be diluted considerably by pooling. Therefore, larger pools could present less risk.

For a reliable assessment of the influence of the pool size, more knowledge would be required on the infective dose in humans, the degree of aggregation of infectivity, its dispersibility, and the pathogen concentrations which can occur in the blood of asymptomatic donors. Calculations about the relation between pool size and transmission risk (Appendix (A) of [1]), assuming that the pathogen would behave like a virus, show that if a recipient requires life-long treatment, a reduction of the pool size would not contribute to minimizing the risk. The current situation is relatively heterogeneous for products on the German market, with different manufacturers, different countries of origin of the starting plasma, various import products and a great variability of the manufacturing methods.

10.1. Effectiveness of the plasma fractionation steps

Usually, infectious material from brains of scrapie or BSE infected hamsters or mice is used to assess the capacity of process steps to remove the vCJD pathogen. The question is to what extent such material is representative of the potential vCJD pathogen in human blood. In a comparative study, no differences in removal of PrP^{Sc} were observed between material from the brain of humans who had developed vCJD, sCJD or GSS, and material from the brain of scrapie infected hamsters [76]. So far, no major differences of pathogen reduction

have been reported when different detection methods (PrP^{Sc} detection versus bioassay) were used [68,77,78]. However, preparation of infectious material from brain can influence pathogen removal: highly purified PrP^{Sc} can aggregate into high molecular fibrils, which may behave differently than dispersed brain material or infectivity in the microsomal fraction [79]. The degree of aggregation is particularly important for pathogen retention in nanofiltration [80] and precipitation and separation by means of centrifugation and depth filtration. It was shown that PrP^{Sc} tends to aggregate in the alcoholic production intermediates during plasma fractionation [81,82]. Despite the above mentioned uncertainties in the interpretation of the experimental data, a reasonably homogenous picture is revealed for plasma fractionation.

Several publications are available for the conventional alcohol fractionation steps of plasma derivatives [68,79,82–86], which state that the pathogen is removed successively from the albumin and immunoglobulin fractions. For coagulation factors, however, such a generalization is far more problematic since individual production processes may differ considerably. This is why the EMEA position paper of 23 June 2004 (EMEA/CPMP/BWP/2879/02) required manufacturers to assess their production methods specifically and to carry out their own experimental trials if suitable published results were not available. PrP^{Sc} reductions by at least 4 log steps have been reported so far for Factor VIII [77,85,87].

10.2. Nanofiltration

Considerable reduction factors are reported by filter manufacturers and plasma fractionators for nanofiltration. However, studies were carried out with differing TSE spiking materials (e.g. fibrillary material, detergent treated material, brain homogenate). The infectious form(s) of the vCJD pathogen is (are) currently still unknown. What the effect of the nanofilters on smaller prion aggregates would be remains open. It is assumed that for prion monomers, no mechanical exclusion by pore size would be given. However, reduction on the basis of other interactions with the filter materials cannot be excluded. The actual benefit of nanofiltration for the removal of vCJD pathogen, therefore, remains fraught with some uncertainty. Until recently, the view prevailed that nanofiltration was not possible with large sensitive molecules such as factor VIII. This option, however, has been implemented by the French manufacturer LFB (pore sizes 35 nm and 15 nm). Since problems might occur that cannot be assessed in laboratory tests, e.g. the development of neoantigenicity, clinical testing should be discussed before marketing authorization. The change in France was effected without clinical trials. However, no additional adverse effects have so far been observed after the change. A detailed discussion of nanofiltration can be found in Appendix (B) of [1].

Hence, several manufacturing steps of plasma products can considerably reduce vCJD infectivity from the starting material, but the extent of this reduction must be further tested and validated. The risk of infectious fractionated plasma products should be markedly lower compared with blood components. The assessment of the safety of recombinant products is not the subject of this report. Therefore, reference is made only briefly to a few aspects. Human plasma derivatives, essentially albumin, may be used as a stabilizer during production of certain recombinant products. In eukaryotic cell cultures, materials of bovine origin are sometimes used. The risk of primary infections with the BSE pathogen must be considered in principle; however, such theoretical risk is minimized [88,89], e.g. by purchasing materials from BSE-free countries. Individual tolerability of different products in the patient and relative frequency of the development of inhibitors must also be considered in the overall assessment of safety. In the past, bottlenecks have existed in the supply of both recombinant coagulation factors and coagulation factors prepared from plasma. With the current state of knowledge, there is no need to advise against the use of plasma derivatives if the indication is established correctly. In hemophilia treatment, decision between coagulation factor products manufactured from plasma and recombination coagulation factors must be considered very carefully, taking into account the situation of the individual patient. A schematic recommendation cannot be given here.

11. Optimal use of blood products

Being "medicinal products from humans," blood products cannot be entirely risk-free, despite the great progress in safety. Critical indication and restrictive administration of blood products are therefore essential to reduce the patients' risk, which is particularly true for a potential transmission of vCJD by donor blood. In the Sanguis Study [90,91], noticeable differences were found in the frequency of transfusion among 43 hospitals from 10 European countries participating in the study. As extreme examples, the preoperative request for provision of RBCC in cholecystectomy was more than ten times that of the actually transfused units, and the frequency of transfusion of hemicolectomy patients ranged between 0% and 79%. Such differences can hardly be explained. Heterogeneous transfusion practice has not changed significantly in the past few years: a more recent Finnish study thus shows that contrary to international recommendation, the median pre-transfusion hemoglobin in transurethral prostate resection was 112 g Hb/l [92]. Various authors have stated unanimously that prospective transfusion criteria and consistent instructions of personnel would lead to a considerable reduction of the consumption of blood components [93,94]. Another possible approach would be autologous blood transfusion, which would avoid any de novo infection relating to allogeneic blood products (including vCJD). Autologous blood transfusions, however, can be performed only in elective surgery with a timely and reliably foreseeable transfusion requirement [95].

Under German EU presidency in 1999, a meeting was held in Wildbad Kreuth [96] with experts in attendance from the EU member states. An assessment of the current situation concerning the use of the most important blood products was elaborated and questions of use, quality management, and economic aspects of transfusion medicine were summarized. It

would be desirable to continue this Kreuth initiative. In regards to therapy with blood components and plasma derivatives, an interdisciplinary working group of the Bundesärztekammer (German Medical Association), summarized basic principles for a clinically indicated use of all important blood products with special consideration of the international literature, national and international consensus conferences and clinical experiences [97]. An essential contribution in Germany has been the requirement laid down in the Transfusion Act of 1998 to establish in all health care facilities a well-functioning quality assurance system for the use of blood products. An appropriately qualified physician responsible for transfusions must be designated and, in addition, transfusion representatives in each clinical unit. This was transposed into the German hemotherapy guidelines [75]. At a European level, too, appropriate recommendations were adopted [98]. Of crucial importance is how these guidelines are implemented and used by hospitals and doctors. It is necessary that such efforts are actively encouraged on the part of the top managers of health care facilities and the health policy makers and recognized by the health care providers who are supposed to finance them.

Optimal use of blood and blood products is an undisputed goal, especially as a safety measure in view of vCJD. Shortly after the first suspected case of transmission was disclosed, the EU Commission called in a "Technical Meeting of Blood Experts related to vCJD transmission" on 20 January 2004 in Luxembourg. One of the statements elaborated by this meeting reads as follows: "There was agreement that optimal use of blood may further reduce the risk of transmission of vCJD by avoiding unnecessary exposure to allogeneic blood transfusion. In addition, avoiding unnecessary transfusion may improve the availability of blood for transfusion; this in turn may facilitate the introduction by Member States of additional donor deferrals if required."

12. Diagnosing vCJD: screening tests

Clinical diagnosis of vCJD can, in principle, be carried out premortem in symptomatic individuals by screening for PrP^{Sc} in the tonsils [27,35], though such a biopsy presents a burden for the patient. At present, solid confirmation of vCJD by histological display of amyloid plaques or detection of PrP^{Sc} in brain material by Western blot is only possible by brain biopsy or post mortem. Clinical diagnosis is a laborious process consisting of various methods and is of subordinate importance for the safety of blood donations. A summary can be found in Appendix (C) of [1].

The development of screening tests is one of the key endeavors for the safety of blood donations. The principal goal of a vCJD screening test is to detect infections as early as possible before onset of initial symptoms in order to prevent possible further transmissions and, if appropriate, to allow therapeutic measures to be taken in an appropriate time frame. Though intensively pursued by a number of groups, so far no concrete success has emerged. The current approaches towards screening tests using blood or other easily accessible body fluids are based either on direct detection of PrP^{Sc} or on the detection of other markers associated with the infection (surrogate markers) [99]. One of the problems with detecting PrP^{Sc} in body fluids is the extremely low concentration at which it may occur in the periphery; estimates expect considerably less than 1 pg/ml PrP^{Sc} in the blood. The most sensitive antigen tests (e.g. for the detection of HBsAg of HBV or p24 of HIV, two proteins with a molecular size similar to that of PrP^{Sc}), after many years of development and improvement, are capable of detecting antigen only at levels of 10 pg and above per ml plasma or serum. In addition, physiological prion protein is present in approx. 10,000-fold excess, which makes the sensitive and specific detection of PrP^{Sc} considerably more difficult. Highly specific so-called "conformational" antibodies (for the recognition of PrP^{Sc} characteristic folding epitopes or conformation epitopes) therefore seem indispensable for a sensitive detection of this protein. The possible use of such an immunoassay (CDI; "conformation dependent immunoassay") for clinical diagnosis of infections is currently under discussion [100].

Research projects are pursuing different approaches to surmounting these limitations, e.g. attempts to increase the tests' sensitivity by means of spectroscopic techniques, enrichment steps to increase the PrP^{Sc} concentration by selective precipitation of PrP^{Sc} through its binding to "ligand" molecules, or cyclical amplification of the pathogen prion protein. The artificial in vitro replication of PrP^{Sc} by means of the PMCA method ("protein misfolding cyclic amplification") [101] has raised high expectations. However, demonstration of the possibility to replicate infectious PrP^{Sc} in one species by a factor of 10^3 [99,102] has not yet led to the development of test systems for routine screening.

The obvious difficulties with the sensitive detection of PrP^{Sc}, the only known specific marker of vCJD infection, have led to the exploration of alternative test concepts. A possible choice would be a screening strategy using single surrogate markers or a combination of them, which could be carried out both at the RNA level (differential display) and at the protein level (proteome analysis). Previously, analyses of differential gene expression in TSE infections have shown that a number of genes are over- or under-expressed in the course of the disease. Several groups have examined to what extent the differential gene expression in the course of the disease could contribute to a better understanding of the infection. Much attention was attracted by the publication of a peripheral marker detectable in blood cells ("erythroid differentiation factor, EDF") [103]. Follow-up tests, however, showed that it is subject to major fluctuations in healthy individuals [104]. Several candidate surrogate screening markers have been published, while proof for these remains to be provided.

Extensive examinations on well-defined populations and acceptable test features (sensitivity, specificity, high throughput) are indispensable preconditions for introducing a screening test, especially in blood donation screening. These conditions have so far not been met by any of the test procedures, and specific criteria should be established for validation of new vCJD tests. Evaluating them in healthy populations,

e.g. blood donors, raises a number of unresolved ethical questions. For instance, how should reactive test results be handled when obtained with the first available test, which cannot be confirmed or clarified by another method?

13. Exclusion of persons from donating blood

Donor selection criteria based on the history of the donor must still be used for risk prevention; an overview can be found in Appendix (E) of [1]. The regulations applicable in Germany have been adopted in Haemotherapy Guidelines [75].

In the past few years it has been discussed whether potential transmission by transfusion could lead to perpetuation of vCJD among humans, even though transmission through the food chain has been stopped, and whether an exclusion of transfusion recipients could essentially influence the course of the vCJD epidemic. In several European countries including the UK, the Netherlands, Switzerland and France—there albeit as early as 1998 under the impression of virus transmissions—the exclusion of transfusion recipients from donating has meanwhile been laid down.

In order to obtain a scientific database, a model calculation based on very pessimistic assumptions was prepared. The epidemiological model describes the spreading of an infection, in this case vCJD, due to blood donations based on the demographic situation in Germany. It assumes that 2,000 individuals were infected by contaminated food during a limited period of 10 years. The total population comprises 80 million people. The parameters for the model were estimated on the basis of four data sets:

1. Donations from 262,071 donors at the blood donation services of DRK (DRK-Blutspendedienst) West Hagen, Germany;
2. 617 controls of a case control study on Creutzfeldt–Jakob Disease at Göttingen University, Germany;
3. age distribution of 1,343 transfusion recipients at the University Hospital of Essen, Germany; and
4. a longitudinal study from Newcastle on the survival of 2,888 patients after a blood transfusion in June 1994.

The age structured model uses 2-month intervals and takes into account the following conditions:

- The mandatory age limit for blood donors is between 18 and 68 years. Each blood donor undergoes an active phase of donor activity the duration of which depends on age.
- The risk of receiving a blood transfusion strongly depends on age and has its peak at approx. 70 years.
- Survival after a blood transfusion also strongly depends on age. The increased mortality rate of transfusion recipients reduces the risk of spreading by blood donations.
- The model takes into account the current mortality rates in the Federal Republic of Germany.
- A mean incubation period of 16 years with a standard deviation of 4 years was assumed for the infection.

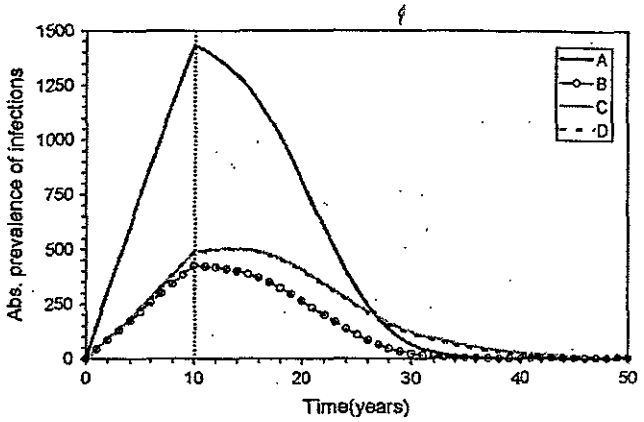

Fig. 3. Prevalence of vCJD. The vertical line shows the end of the phase of introduction of the infection by food. The curves show prevalence with the denominator of 80,000,000 German population, for the following cohorts: Curve A: Non-recipients (individuals *without* transfusion history, infection is therefore only possible by food). Curve B: Recipients (individuals *with* transfusion history), if transmission by transfusion is excluded (infection risk 0%, therefore—as in Curve A—infection possible only by food). Curve C: Recipients, if infected blood donations always lead to infection of recipients (infection risk 100%). The difference between Curve C and Curve B shows that the majority of infections was not caused by blood donations but by food. Curve D: as Curve C, but excluding donors with transfusion history. Reproduced from Fig. 2A in "How much would the exclusion of transfusion recipients from donating blood reduce the spread of vCJD?" *Emerging Infectious Diseases*, Vol. 13 No. 1, 2007.

- The model permits exclusion of donors with a history of blood transfusions. It is assumed that 95% of the donors with a history of blood transfusions can be excluded.

Fig. 3 shows the absolute infection prevalence as a function of time predicted by the model. Prevalence increases during the 10-year period of food related infection and leads to a maximum of 1,434, infected individuals in the portion of the population without transfusion history (Curve A). In the portion of the population with transfusion history, depending on whether no risk of infection is assumed (0%, Curve B) or, in the most unfavorable case, an absolute infection risk is assumed (100%, Curve C) by blood donations from infected donors, 426 or 504 infected individuals, respectively, are to be expected. Maximum prevalence in the German population is 1,860 or 1,921 infected individuals respectively, corresponding to approx. 24 infected individuals per 1 million inhabitants. (The maximum value of 1,921 is slightly smaller than the total of 1,434 plus 504, since the maximum values of the individual curves are reached at different times.) The majority of infections caused by transfusions cannot be prevented by the exclusion of donors with a transfusion history, since they were infected by blood from food infected donors without a transfusion history. Thus, an exclusion of transfusion recipients would bring about only a minor contribution to prevention (Curves C and D can hardly be distinguished).

Given the initial rate of introduction of the infection, no further spreading occurs after that period of time, and, due to decreased life expectancies of vCJD infected individuals, the prevalence during the subsequent 20–30 years has a tendency

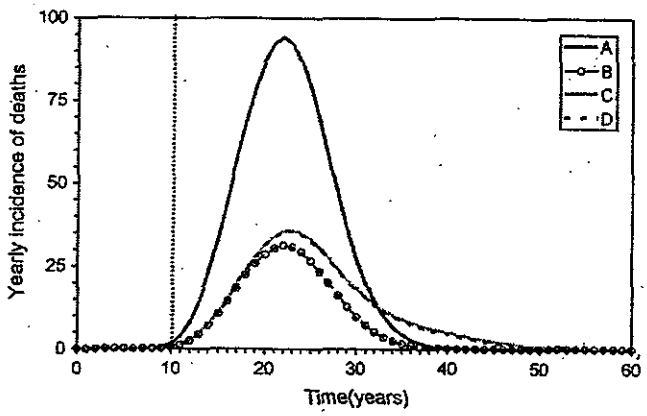

Fig. 4. Mortality from vCJD. The vertical line shows the end of the phase of introduction of the infection by food. The curves show incidence of deaths (Fig. 4) with the denominator of 80,000,000 German population, for the same cohorts A–D shown in Fig. 3. Reproduced from Fig. 3A in "How much would the exclusion of transfusion recipients from donating blood reduce the spread of vCJD?" *Emerging Infectious Diseases*, Vol. 13 No. 1, 2007.

towards zero again. Even if it is assumed that infected blood donations will always lead to infection of the recipient (infection risk = 100%), no further spreading occurs. In addition, the decrease in prevalence is only delayed due to the incubation period of the individuals infected (Curve C). An exclusion of transfusion recipients, even in the latter most pessimistic scenario, can bring about only a minor contribution to prevention (Curve D). This is also shown in Fig. 4, in which the annual incidence of deaths due to infection is shown. The maximum number of vCJD associated deaths occurs not before 23 years after the beginning of the onset of infection due to the long incubation period.

Fig. 5 compares the incidence of deaths of individuals infected by food (Curve E) with those that may be caused by blood donations with maximum risk of infection (Curve F). Due to the incubation period, transfusion associated deaths

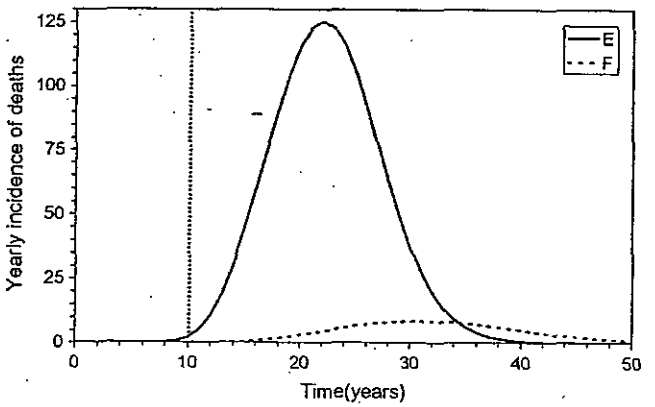

Fig. 5. Curve E: Deaths due to vCJD infections that could only be caused by food (totals of Curves A and B in Fig. 2). Curve F: Deaths due to vCJD caused by blood from infected donors (infection risk 100%) (difference between Curves C and B in Fig. 2). The vertical line marks the end of the phase of infection by food. Reproduced from Fig. 4A in "How much would the exclusion of transfusion recipients from donating blood reduce the spread of vCJD?" *Emerging Infectious Diseases*, Vol. 13 No. 1, 2007.

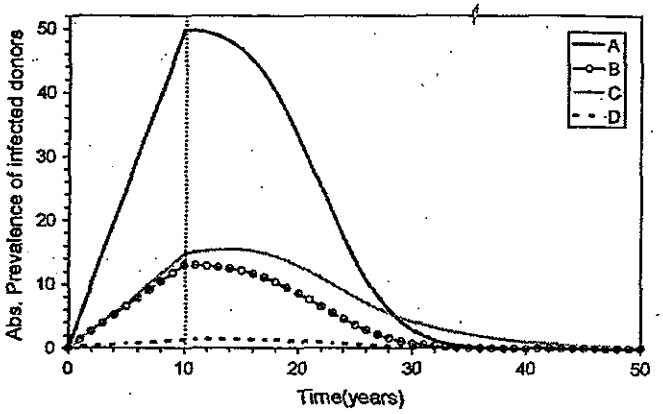

Fig. 6. Curve A: Prevalence of infected donors *without* transfusion history (infection possible only by food). Curve B: Prevalence of infected donors *with* transfusion history, if transmission by transfusion could be ruled out (infection risk 0%, therefore—as in A—infection possible only by food). Curve C: Prevalence of infected donors *with* transfusion history, if it is assumed that infected blood donations always lead to infection (infection risk 100%). The difference between Curve C and Curve B show that the majority of infections are not caused by blood donation but by food. Curve D: As Curve C, but excluding donors with transfusion history. This curve shows 5% of the donors with transfusion history, who were not excluded according to the model assumptions. The vertical line shows the end of the phase of infection by food. Reproduced from Fig. 5A in "How much would the exclusion of transfusion recipients from donating blood reduce the spread of vCJD?" *Emerging Infectious Diseases*, Vol. 13 No. 1, 2007.

occur markedly later than deaths caused by food infections. Within the displayed 50 years, 172 transfusion associated deaths have to be expected. During this period, however, a maximum of 15 cases could be prevented if donors with a transfusion history were excluded, equivalent to 1 case in 3–4 years. Out of the 2,000 individuals infected by food, we expect 1,557 vCJD cases if the infection risk of infected donors is 0%, and 1,729 cases if the infection risk is 100%. If approx. 20% of the donors were excluded, less than 1% of the cases would be prevented. Fig. 6 explains why the exclusion of donors with a transfusion history only slightly influences the incidence of deaths:

- The majority of the infected donors were infected by food and reveal *no* transfusion history (Curve A). This group is not covered by the exclusion criterion "donors *with* a transfusion history" and is able to continue to transmit the disease.
- Infected donors *with* a transfusion history can be excluded, but represent only a minor portion of infected donors (Curve B or C, respectively). The portion of donors infected by transfusions is very small (difference between Curve C and Curve B).

The assumptions chosen here present a considerable overestimation of the real risk of infection in Germany. Since an infection introduced by food cannot be sustained in the population, there is no further long-term risk after this route of transmission has been interrupted. Because of the low prevalence of approx. 24 infected individuals per 1 million (see above), linear reduction can be performed on predicted

developments if markedly lower prevalences are assumed. The actual prevalence of individuals infected by food in Germany is probably lower by at least a factor of 10. Therefore, the above mentioned figures can probably be reduced by the corresponding factor. A detailed description of the model with all parameters and figures for the data sources on which this calculation is based has been published [105].

14. Impact of deferrals on the blood supply

Before introducing any donor deferrals, their effects should be quantified and a risk/benefit assessment (safety vs. blood supply) performed. It can be added to the discussion of the above model that an exclusion of transfusion recipients would not have prevented the three probable transmissions in the UK, since those donors were not transfusion recipients themselves. Moreover, it can be expected that an undetermined number of donors would not remember transfusions and continue to donate blood. On the other hand, as the French example shows, an exclusion of transfusion recipients is possible in principle, but would entail serious problems and disadvantages, would require major efforts and could therefore only be implemented over a longer period of time.

Examples from Germany may illustrate the impact of new exclusion criteria in general. With the 2000 update of guidelines, a rise of Hb limit for men from 125 to 135 g/l was introduced, which had a serious effect on the donor population. The deferral rate (Table 5) initially rose by approx. 2.5%, and after the male donors with Hb between 125 and 135 g/l had been deferred, decreased again to its initial level.[9] Deferral rates tended to be rising from 322,312 in 2002 to 345,906 in 2003 (8.25% vs. 8.87% of the population prepared to donate). Since winter 2000/2001—despite intensive encouragement to donate blood—the demand for blood components, especially RBC, has not always been met so that, e.g., planned operations have to be postponed. Also, stricter donor exclusion criteria for the sake of improved safety may lead to a loss in donors, as anti-HBc testing [106] has led to permanent exclusion of approx. 0.6–1% of blood donors. New reasons for exclusion frequently are not understood by those concerned and require great educational efforts. As experience with the deferral due to a cumulative stay in the UK of more than 6 months between 1980 and 1996 has shown, considerable uncertainties remain despite numerous dialogues due to the lack of possibility to explore the donor's individual risk or to obtain a confirmatory/exclusion test. Exclusion of transfusion recipients could even be perceived as a signal that, despite all efforts, blood supplies are not sufficiently safe.

To secure blood supplies, new donors would have to be recruited to a considerable extent. According to the German data on epidemiology collected by the Robert Koch-Institut, the prevalence of virus infections in new blood donors is higher than in long-term donors. While special promotion campaigns (cf. bone marrow/stem cell donors for children with leukemia) motivate many people to donate in the short-term, it is the continued reliability of donor preparedness in connection with the constantly required readiness to act that is important in blood donor promotion campaigns. The following possible approaches could secure continued donor preparedness and the supply of blood components:

- Economical use of blood and blood products: All measures that result in optimal use of blood and blood products will not only minimize the possible transmission risk but will also contribute to safeguarding the supply of blood and blood products. The activities in Germany and the European Union have been described above.
- Sustained recruitment measures: The GRC blood donation services are currently spending approx. €20 million a year on maintaining their existing donor base. Additional recruitment campaigns (approx. €3 million annually) have been aimed at the approx. 1–2% donor increase required to compensate for the annual rise in demand due to the increasing average age of the population. The GRC has been able to fulfill this goal with a relatively low budget, since advertising space in various media has generously been made available free of charge. In the case of donor exclusion due to transfusion history, just below 18,000 additional donors per month would be needed in the first half-year, and more than 11,800 additional donors per month in the second. Altogether, approx. 4 first-time volunteers would have to be recruited for each deferred donor (compare Appendix (E) of [1]). Such an additional recruitment campaign requiring more financial means could no longer be carried out by the blood donation services.
- Increasing the social prestige of the blood donors: It might be helpful to provide donors with a non-material reward in the form of increasing the social prestige attached to the act of giving blood. A professional study should explore the possibilities and develop suggestions for raising the esteem in which blood donors are held.

Table 5
Main reasons for deferral according to an analysis by the Institut für Transfusionsmedizin (Institute for Transfusion Medicine) Münster of the German Red Cross blood donation service West in 2004 (in % of volunteer donors)

Hemoglobin	2.21%
Operations, interventions	1.30%
Blood pressure	1.17%
Infectious diseases	1.24%
Suspected/risk of infection	0.78%
Current donation situation (e.g. unease, fear, communication problems)	0.73%
Medication	0.72%
Traveling	0.28%

According to statistics from the German Red Cross blood donation services, the deferral rate among all volunteer donors in 2003 was 8.87%. There is a strong fluctuation in the deferral rate between the various blood donation services, which ranged

[9] German Red Cross (GRC) blood donation centre West, figures from North Rhine-Westphalia.

from 5–14% and is now 7–12%. No correlation can be detected between the deferral rate and the degree of conurbation. These differences can certainly not solely be explained by differences in the donor population of the German Red Cross blood donation services. An improvement could be the introduction of uniform interpretation aids throughout Germany.

15. Summary and conclusions

So far 162 cases of vCJD in the UK, 20 in France, 4 in Ireland, 2 in the Netherlands, 2 in the USA, and one case each in Canada, Italy, Japan, Portugal, Saudi Arabia, and Spain have been observed. Twenty-seven of these patients resided outside the UK and had not spent considerable time in the UK; a connection with stay in the UK is questionable in the case in Japan. It must not be ruled out that vCJD will be diagnosed in more countries. Recent model calculations in the UK [50] have resulted in lower than previously published estimates of the overall number of clinical vCJD cases, however, with considerable confidence intervals. A fundamental assumption is that new infections from the food chain have been effectively stopped. On the basis of new estimates, the number of up to 600 cases of vCJD for Germany indicated in the report of this group in 2001 can be considered as too pessimistic.

Three cases published in the UK since 2004 must be regarded as evidence for the transmissibility of the vCJD pathogen by blood. One of the recipients died from an unrelated disease. However, autopsy revealed the vCJD pathogen in the spleen and lymph nodes, pointing to a subclinical or not yet symptomatic infection. Unlike all other previously observed vCJD cases, this patient was heterozygous M/V at codon 129. This observation and the results from a serial investigation of appendix tissue in the UK could indicate that there are a considerable number of infected persons who might not develop vCJD, or in whom its manifestation is delayed. At present, it is not possible to ascertain whether infectivity is present in the blood of these persons, and if so, at what time and to what extent. Precautionary measures should therefore not be based exclusively on the number of already manifested vCJD cases and the forecast of future numbers of cases derived thereof.

A possible transmission of the vCJD pathogen by plasma products still cannot be entirely ruled out, but it seems unlikely since various experimental systems have shown that prions are largely removed during the manufacture of these blood products. Examining the effectiveness of these steps, however, should be continued in a product-oriented manner. A Note for Guidance was published in 2004 by the European Medicines Agency (EMEA) for this purpose [88].

Transmissibility of vCJD by transfusion had been assumed already in 2001; precautionary measures for minimizing the risk had been taken. As an additional measure, the exclusion of transfusion recipients from donating blood has to be considered in order to break a hypothetical chain of further spread and possible perpetuation of vCJD by blood products. Such exclusion has been introduced in the UK, the Netherlands, Switzerland, and as early as 1998—to prevent viral transmissions—in France. A model calculation took these considerations into account; to be on the safe side, still a worst case scenario was dealt with. For Germany, the group concluded that such exclusion is not warranted. The evaluation of the potential gain in safety regarding vCJD, based on the above mentioned model calculation, was not considered to outweigh the drawbacks of such a measure.

The secondary route of infection by blood could largely be stopped as soon as a suitable test could be introduced into routine donor screening. No such test is currently available; developing and optimizing test methods should have high priority.

Acknowledgements

The excellent secretarial support by Mrs. Helene Knöß is gratefully acknowledged.

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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	総合機構処理欄
一般的名称	－		研究報告の公表状況	Current opinion in hematology (United States) May 2007, 14 (3) p210-4.	公表国 米国	
販売名（企業名）	－					

研究報告の概要

英国で白血球除去が導入される前に赤血球製剤の輸血により、変異型クロイツフェルトーヤコブ病（vCJD）の伝播が3例報告されている。
げっ歯動物の伝染性海綿状脳症（TSE）の実験では、赤血球製剤の感染性は赤血球自体とは無関係であり、感染性は残留白血球や血漿中の他の成分と関連することが示された。
ハムスターの異常プリオンを添加した白血球除去ヒト赤血球の感染性は 0.01％まで除去されたと報告もあり、これは感染性が赤血球によるものではない、あるいは赤血球と感染物質が結合していてもその結合は緩く、ろ過プロセスによって除去されることを示している。
vCJD の原因物質がヒト赤血球と結合しないことを確認できたならば、血中の PRPsc の適切なスクリーニング検査がない現状においては、vCJD 発生国の輸血サービスは赤血球製剤を輸血する前に洗浄またはろ過処理して感染性除去を行うことが賢明である。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
英国における輸血による vCJD 感染例は白血球除去を行っていない赤血球製剤によるものであるが、赤血球自体には感染性はなく、ろ過により除去できるとの情報である。 現時点まで血漿分画製剤からの vCJD 伝播の報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。	今後とも vCJD に関する安全性情報等に留意していく。

Prion protein and the red cell

David J. Anstee

Purpose of review
This review focuses on transfusion-transmission of variant Creutzfeldt-Jakob disease by red cell preparations.

Recent findings
Recently, three cases of probable transmission of variant Creutzfeldt-Jakob infectivity by transfusion of red cell preparations have been described in humans. Experiments on transmissible spongiform encephalopathies affecting rodents have led to the conclusion that infectivity in red cell preparations is not bound to the red cells themselves but contained within the suspending medium from which it can be removed by filtration.

Summary
Red cell preparations are the main transfusion product provided by blood services. If experiments demonstrating significant removal of rodent transmissible spongiform encephalopathy infections by filtration of red cell preparations are applicable to variant Creutzfeldt-Jakob in humans then a method for rendering human red cell preparations safe for transfusion is provided.

Keywords
bovine spongiform encephalopathy, normal prion protein, abnormal (infectious) prion protein, transmissible spongiform encephalopathy, variant Creutzfeldt-Jakob disease

Curr Opin Hematol 14:210–214. © 2007 Lippincott Williams & Wilkins.

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Current Opinion in Hematology 2007, 14:210–214

Abbreviations

BSE	bovine spongiform encephalopathy
GPI	glycosylphosphatidylinositol
PRPc	normal prion protein
PRPsc	abnormal (infectious) prion protein
TSE	transmissible spongiform encephalopathy
vCJD	variant Creutzfeldt-Jakob disease

1065-6251

Introduction

Variant Creutzfeld-Jakob Disease (vCJD) was described in the UK in 1996 [1]. The emergence of this novel form of CJD is most probably related to the ingestion of food products obtained from cattle with bovine spongiform encephalopathy (BSE) [2,3]. From the outset the possibility was considered that passage of infectivity from the gut to the brain in affected individuals could involve blood. Therefore, transfusion services were alerted to the potential for transmission of vCJD to a patient by transfusion of blood components from a donor in the preclinical stages of disease. Consequently, precautionary measures were taken in the UK to minimize this risk, in particular leucodepletion, sourcing plasma for fractionation from non-UK donor populations, sourcing fresh frozen plasma for children born after 1 January 1996 (and therefore not exposed through diet) from non-UK donor populations and deferral of blood donors who had themselves been transfused [4]. Sourcing sufficient red cell and platelet components outside the UK is not feasible. The first cases of probable transfusion–transmission emerged in late 2003 and three probable transmissions, all linked to red cell preparations transfused before the introduction of leukodepletion, are now recorded [5,6,7*,8**].*

The infectious agent

There is a large body of evidence suggesting the infectious agent causing BSE and vCJD is an abnormal conformer of the prion protein [9]. Recently, evidence has emerged suggesting that retroviral infection can increase the release of infectious prions from cells and may be an important cofactor in the spread of infection [10*].

Normal prion protein (PRPc) is a glycosylphosphatidylinositol (GPI)-linked protein expressed at cell surfaces of many tissues. It is a glycoprotein rich in α-helix. The function of PRPc is unclear, although recent studies suggest a role in self-renewal of haemopoietic progenitor cells [11*]. The infectious prion protein (PRPsc) is an abnormal conformer of PRPc in which the α-helical regions become predominantly β sheet. This change in secondary structure alters the properties of the protein so that PRPsc has a greater propensity to aggregate. Aggregates of PRPsc accumulate within cells in the brain of

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affected individuals creating the toxic environment that ultimately results in spongiform encephalopathy. Size fractionation suggests the most infectious prion particles comprise aggregates of 14–28 molecules [12]. Once a small amount of PRPsc is ingested it can associate with PRPc from the affected individual and convert this PRPc to PRPsc creating an autocatalytic effect, which greatly increases the amount of PRPsc in the affected individual. This autocatalytic effect has been reproduced *in vitro* in a hamster model [13].

Normal prion protein is essential for the disease process

PRPc must be available for prion disease to occur [14,15,16•]. Furthermore, mice engineered to translate PRPc without a GPI anchor accumulate PRPsc in the brain, blood and heart but do not develop clinical scrapie [17,18]. These results indicate that membrane tethering of PRPc is essential for disease progression. GPI-linked proteins frequently occupy lipid rafts in the plasma membrane. In cultured adult sensory neurones PRPc leaves lipid rafts to recycle between the cell surface and recycling endosomes in a time scale of minutes [19]. The mechanism whereby PRPc is converted to PRPsc is not fully understood but may occur at the cell surface when PRPc leaves its lipid raft prior to endocytosis [20]. After endocytosis, PRPc goes to recycling endosomes [21] while PRPsc trafficks to lysosomes [22]. Passage of PRPsc to lysosomes can be via multivesicular bodies from which small vesicles 40–100 nm in diameter (exosomes) rich in GPI-linked proteins bud off and are released from the cell. These exosomes can contain PRPsc and have the potential to transfer infectivity from one cell/tissue to another [23].

Transfer of infectious prion protein from gut to brain

After peripheral infection, PRPsc accumulates and replicates in the lymphoreticular system, particularly the spleen and lymph nodes, prior to neuroinvasion and disease. The process by which PRPsc travels from gut to the lymphoid organs may occur via the blood through bone marrow-derived dendritic cells, which pick up PRPsc in the gut and transport it directly to the lymphoid tissues [24]. In rodents, follicular dendritic cells (FDCs) found in the germinal centres of lymphoid organs are major sites of PRPsc accumulation and the rate of transfer of PRPsc from lymphoid tissue to sympathetic nerves is likely determined by the relative positioning of FDCs and sympathetic nerve endings [9,25].

Infectious prion protein in blood

The foregoing discussion describes a process whereby infectivity (PRPsc) in the gut passes via the blood to the spleen and lymphoid cells and thence to the brain by way of antigen-presenting cells capable of taking up and replicating PRPsc. If the antigen-presenting cells come into contact with other blood cells whilst in transit from gut to lymphoid tissues or process PRPsc in a manner which results in the generation of exosomes containing PRPsc it is possible infectivity could transfer to other cells in blood. The cycle of PRPsc replication could continue within those other blood cells that have the necessary intracellular organelles, and those cells without the necessary machinery for recycling PRPsc like red cells may act as passive carriers of infectivity. There is considerable evidence for the occurrence of exosomes in human blood [26] and that they can derive from platelets [27] and reticulocytes [28] as well as from circulating dendritic cells [29]. Furthermore, transfer of GPI-linked proteins CD55 and CD59 from transfused red cells to the red cells of a patient with paroxysmal nocturnal hemoglobinuria has been demonstrated *in vivo* [30]. In this context it is interesting to note that exosomes containing HIV-1 released from immature dendritic cells were found to be 10 times more infective of CD4+ T cells than cell-free virus particles [31].

Exosomes do not provide the only hydrophobic environment in plasma. Recently, evidence has been presented [32•] showing that brain-derived PRPsc binds with high affinity to apolipoprotein B, the major component of very low density and low-density lipoproteins (VLDL and LDL) in plasma.

Infectivity in red cell preparations used for transfusion

There is persuasive evidence [8••] that transfusion of red cell preparations from donors who subsequently developed vCJD has transmitted the disease to three recipients. In each of these cases, the transfusions took place before leucodepletion of red cell preparations was introduced in the UK. Leucodepletion of 450 ml whole blood collected from scrapie-infected hamsters removed 42% of the total infectivity [33]. Whether or not a similar reduction in infectivity is achieved by leucodepletion of human blood is unknown. More relevant is whether or not leucodepletion of human blood is sufficient to prevent transfusion–transmission of vCJD. The follow-up of recipients of leucodepleted red cell preparations from donors who subsequently developed vCJD will provide information of relevance to this question [8••]. The leucodepletion process itself does not appear to result in increased numbers of leucocyte microvesicles that may carry infectivity [34] but would probably not remove exosomes. Given the uncertainty concerning the effectiveness of leucodepletion in removing infectivity from human blood, attention has turned to the possibility of employing filters, which selectively remove PRPsc. Sowemimo-Coker *et al.* [35] filtered 300 ml red cells from 500 ml anticoagulated whole blood collected from scrapie-infected hamsters. They report transmission of

disease to six of 43 hamsters receiving unfiltered red cells but none of 35 hamsters given filtered cells. Gregori *et al.* [36•] report removal of all but 0.01% infectivity from leucodepleted human red cells spiked with scrapie from hamster brain. These studies indicate that infectivity is not intrinsic to red cells or that if infectivity is associated with red cells it is loosely bound and removed by the filtration process. These data, if transferable to the human situation, provide a means of securing the safety of red cell transfusions in countries where the population has been exposed to BSE. Neither study, however, precisely mimics the human situation and so it is necessary to consider the suitability of hamster scrapie as a model for BSE and the similarity between the blood cells of hamsters and humans.*

Of hamsters and men

As it is extremely difficult to design experiments that directly address the biology of vCJD in human blood, most of the data available relate to animal red cells and transmission of scrapie rather than BSE. Whole blood transfusions between sheep have demonstrated transmission of BSE but these experiments have not yet been extended to transfusion of the individual components of blood [37].

As described above, available evidence suggests that prion disease cannot develop in the absence of PRPc. It is therefore reasonable to ask what is the distribution of PRPc in human blood cells and how does it compare with PRPc distribution in blood cells of animals used for investigation of blood-borne TSE infectivity, since differences in PRPc expression may occur and be relevant to disease progression. Holada and Vostal [38] report flow cytometric experiments demonstrating low levels of PRPc on human red cells and absence of PRPc from hamster red cells. Experiments of this type, which utilize a single monoclonal antibody to PRPc, may give erroneous information if the relevant PRPc epitope is not accessible on the cell type examined because of differences in posttranslational modifications like glycosylation [39]. If hamster red cells differ from human red cells in lacking PRPc expression, however, are hamsters a relevant model with which to study the infectivity of human red cells?

If hamster scrapie strain 237K PRPsc does not bind to human red cells does this necessarily mean that BSE/vCJD PRPsc does not bind either? Nishina *et al.* [40•] reported that diglycosylated hamster brain PRPc is required for the amplification of hamster PRPsc strain 237 *in vitro* whereas unglycosylated mouse brain PRPc is required for the amplification of RML PRPsc, a clear indication that different sources of PRPsc have different requirements for glycosylation of PRPc. Earlier work [41] also demonstrated that the glycosylation profile of PRPc can influence the amount of PRPsc bound.

The same protein can have different glycosylation profiles in different tissues from the same animal [42,43]. Clearly, such tissue-specific differences in glycosylation of PRPc could result in tissue-specific differences in binding and replication of PRPsc and account for heterogeneity of PRPc isoforms observed in different regions of mouse brain and for different patterns of PRPsc deposition by different PRPsc strains [44].

These considerations lead to the conclusion that expression of PRPc on a given cell or tissue is not, of itself, an indication of susceptibility to PRPsc binding. Consequently, the glycosylation profile of PRPc on red cells may influence the ability of different strains of PRPsc to bind to red cells and may account for the lack of PRPsc binding observed in animal experiments described above. The same reasoning applies to the interpretation of animal experiments examining the infectivity of blood platelets. Hamster platelets lack PRPc whereas human platelets express PRPc at high levels [45]. Platelets were found to lack infectivity in the blood of hamsters infected with hamster scrapie [46]. The glycosylation profile of the complement regulatory protein CD59 on human red cells and platelets has been determined in detail. The protein on both cell types is extensively glycosylated but the glycosylation profiles of the protein on the two cell types are distinct [43]. If PRPc on red cells and platelets is glycosylated in a similar manner this may account for absence of PRPsc binding because large N-linked oligosaccharides at Asn181 and Asn197 could shield large parts of the surface of the prion protein and sterically hinder protein–protein interactions [47]. This could also explain why murine red cells which express PRPc [38] lacked infectivity when derived from animals infected with mouse-adapted vCJD [48]. Nevertheless, it would be prudent to investigate binding of BSE PRPsc to human red cells and platelets before assuming that these cells do not carry vCJD infectivity, since the glycosylation profile of a protein can differ between species [49,50].

Human red cell PRPc and hamster brain PRPc may differ in the structure of the GPI anchor. On human red cells, GPI-linked proteins CD59 and acetylcholinesterase are unusual in that the GPI anchor is palmitoylated in a way that renders it resistant to phospholipase C [43,51]. If as Rudd *et al.* [43] point out this is likely to be a feature of all GPI-linked proteins on the red cell, then red cell PRPc would have the same anchor. The GPI anchor found on PRPc from Syrian hamster brain is not palmitoylated in

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this way [47]. This difference may influence the location of PRPc in lipid rafts and thence accessibility to PRPsc [20,52].

Finally, there is also the possibility that human red cells could bind PRPsc independently of PRPc. PRPsc binds with high affinity to plasma lipoproteins [32•]. Plasma LDLs have been reported to bind red cells, albeit with low affinity [53].

Conclusion

Recent reports show there is a high probability that human red cell preparations have transmitted vCJD. Experiments carried out with rodent TSEs indicate that infectivity in red cell preparations is not associated with the red cells themselves but with other constituents of the product such as residual leukocytes and plasma. Lack of intrinsic red cell infectivity may result from posttranslational modifications of the structure of red cell PRPc which prevent PRPsc binding. If it can be shown that the causative agent of vCJD fails to bind human red cells and in the absence of a suitable screening test for PRPsc in blood, it may be prudent for blood services in countries where vCJD occurs to consider processing red cell preparations by washing or filtration to remove fluid phase infectivity prior to transfusion.

Acknowledgements

The author is grateful to Dr P. Hewitt and Dr G. Mallinson for help and advice during the writing of this paper. This work is funded by the UK Department of Health.

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Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 291).

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研究報告の概要

○輸血と変異型クロイツフェルト・ヤコブ病
変異型クロイツフェルト・トヤコブ病(vCJD)は血液を介して感染する可能性がある。血液成分を介した二次感染を予防するため、複数の国で輸血歴のある供血者の除外が開始されている。Dynamic age-structured modelを用いて、この措置の効果を検討した。これは1) 供血者の行動、2)CJDの症例対照試験、3)受血者の年齢分布、4)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。当該モデルから、食肉からヒトに伝播したvCJDは、輸血のみにより感染が拡大する可能性はないこと、また、輸血歴のある供血者を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。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
血液を介したvCJD感染予防の目的で行われている輸血歴のある供血者の除外措置の効果を検討したところ、食肉からヒトに伝播したvCJDは、輸血のみにより感染が拡大する可能性はないこと、また、輸血歴のある供血者を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との報告である。	日本赤十字社は、輸血感染症防止のため輸血歴のあるドナーを無期限に献血延期としている。vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より英国滞在歴1日以上の方からの献血を制限している。さらに、血液製剤の保存前白血球除去を順次導入し、平成19年1月16日からは全ての輸血用血液に保存前白血球除去を実施している。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。

Blood Transfusion and Spread of Variant Creutzfeldt-Jakob Disease

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Variant Creutzfeldt-Jakob disease (vCJD) may be transmissible by blood. To prevent secondary transmission through blood components, several countries have started to exclude as donors persons who have received a blood transfusion. We investigated the effectiveness of this measure by using a dynamic age-structured model. It is the first such model based on epidemiologic data: 1) blood donor activities, 2) a case-control study on CJD, 3) age distribution of recipients, and 4) death of recipients of blood transfusions. The model predicts that an infection like vCJD, which has been introduced into the population by the alimentary route, could not become endemic by transfusion alone and that only <1% of cases would be avoided by excluding from blood donation those persons who have received a transfusion.

Recent studies of variant Creutzfeldt-Jakob disease (vCJD) indicate that this disease is transmissible by blood. One case of probable transfusion-transmitted vCJD infection has been reported, and 1 case of subclinical infection has been detected (*1,2*). On February 9, 2006, a third case was announced by the UK Health Protection Agency (www.hpa.org.uk/hpa/news/articles/press_releases/2006/060209_cjd.htm). Each of the 3 patients had received a blood transfusion from a donor who subsequently developed clinical vCJD, which indicates that transfusion caused the infection. However, a policy to exclude potential donors who had received a transfusion would not have prevented at least the first 2 cases because the corresponding donors had not received any blood transfusion. Diagnostic tools to detect prions in blood are under development (*3*), but no routine test for the presence of the infectious agents of vCJD is available. Therefore, the questions arise as to whether an infection like vCJD could become endemic through blood donation alone and to what extent exclusion of potential donors with a history of transfusion would influence the transmission of such an infection (i.e., how many deaths due to the infection could be prevented?). The following mathematical model is the first to address these questions on the basis of epidemiologic data and realistic and epidemiologically justified assumptions.

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Methods

Model Structure

Figure 1A shows the transitions of a person through the basic states of potential donor activities and receipt of blood transfusion. After birth a person is in the state of not having received any transfusion and not yet being an active donor (S_{00}). The first index refers to the person's state as a transfusion recipient; the second index, to the person's status as a donor. Persons in state S_{00} can change to state S_{01} by becoming a donor or to state S_{100} or S_{101} by receiving a blood transfusion. The third index indicates whether a person with a transfusion history can actually be identified and excluded from donating blood (deferred) (index 1) or not (index 0). The states S_{111} and S_{110} can be reached by either transfusion recipients who start donating blood or active donors who receive a blood transfusion. Blood donors who become inactive are transferred into the states of ex-donors S_{02} and S_{12}, depending on their transfusion history. Ex-donors can also become transfusion recipients; i.e., they are transferred from S_{02} to S_{12}. Donor exclusion transfers a certain proportion of transfusion recipients into

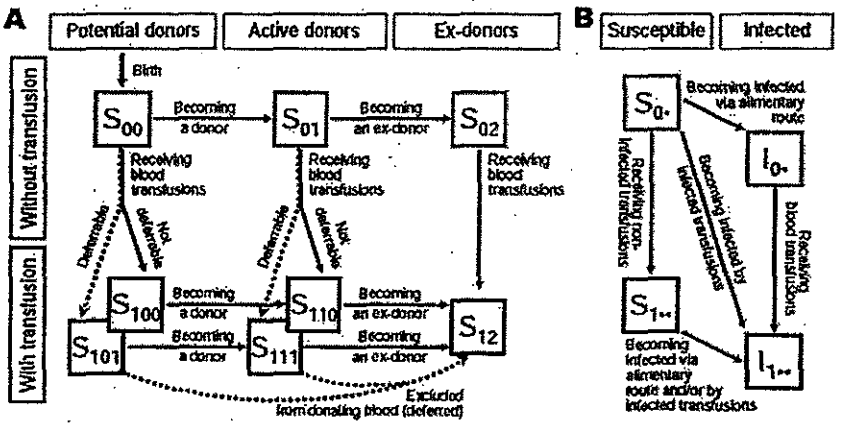

Figure 1. A) States and transitions for the model of blood transfusion in the absence of an infection. B) Routes of infection. The arrows representing deaths out of all states are omitted. Paths of donor exclusion are plotted by dotted arrows. S_{00}, nonrecipients who do not donate; S_{01}, nonrecipients who donate; S_{02}, nonrecipients who are excluded from donating; S_{100}, recipients who do not donate; S_{101}, recipients who become excluded from donating; S_{110}, recipients who donate; S_{111}, recipients who become excluded from donating; S_{12}, recipients who are excluded from donating. Indices replaced by a dot (panel B) represent all other possible states (e.g., $S_{0\cdot}$ represents S_{00}, S_{01}, or S_{02}).

the state of ex-donors. For all susceptible states, Figure 1B shows the transitions to the corresponding infected states. Table 1 provides a list of all input parameters together with descriptions and sources. The details of the model with all the numerical parameter estimates and the equations are given in the online Appendix (available from www.cdc.gov/ncidod/EID/13/1/89-app.htm). The computer program is available upon request. This article summarizes the major features of the model, the data sources, and the estimation of the model parameters.

Demography

To simplify the model, we did not attempt to describe the demographics of the population during the next 150 years. Doing so would involve predicting changes in rates of birth, death, and immigration. It is assumed that in the absence of infection, the population is demographically stationary. We assumed a constant inflow of newborns and an age-specific death rate. The latter was estimated as a weighted mean of the age-specific female and male death rates. Because this study was initiated in Germany, we used the corresponding demographic data. To start the simulation in a demographically stationary state, the model was run for 100 years without infection. Thus, the age distribution of the population was identical to the life table of Germany 2002/2004 averaged over both sexes (www.destatis.de/download/d/bevoe/sterbet04.xls).

Modeling Blood Donors

Blood donors in Germany are ≥18 and <68 years of age. The rates for becoming a new donor and terminating the period as an active donor are age dependent. The corresponding parameters were estimated by using data from 262,071 donors registered with the German Red Cross

Table 1. Summary of input parameters for the model*

Parameters	Description	Source
Age-specific mortality rates	U-shaped, with minimum at age 10.	Federal Statistical Office of Germany
Donor recruitment	Donors ages 18–67 y. Maximum recruitment rate at age 18, lower plateau ages 25–50; further decrease until age 67.	Age-distribution of first-time donors at DRK Blood Service and age structure in population
Proportion of donors	3% of population.	DRK Blood Service West
Duration as active donor	Donors ages 18–40 y, mean duration as active donor 10–14 y, decreases linearly to 0.	Age distribution of active donors at DRK Blood Service West, by age at first donation
Risk of receiving transfusions	Bimodal, with peaks for newborns and aged persons. Multiple transfusions possible.	Data collected from 4,867 patients March 2003, University Hospital Essen, Germany
Transfusion-associated risk for death	Increases according to a sigmoid function, ≈17% at birth to ≈48% in old age. For those with transfusion-associated risk for death, life expectancy is ≈2.5 years at birth and decreases to ≈0.5 y in old age.	Follow-up of ≈3,000 transfusion recipients for ≈7.5 y in Newcastle, UK (*4*)
Alimentary infection	Constant over an initial period of 10 y.	Arbitrary assumption
Incubation period†	Gamma distributed with mean 16 y, SD 4 y. Sensitivity analysis with mean = 50 y and same coefficient of variation.	Models fitted to the UK incidence of vCJD (*5,6*)
Donor exclusion	Either 0 or 95% of those with transfusion history.	Arbitrary assumption

*DRK, German Red Cross; SD, standard deviation; vCJD, variant Creutzfeldt-Jakob disease.
†Time between infection and death, i.e., duration of infection.

(DRK) Blood Service West in Hagen, Germany, including age, sex, age at first donation, number of donations, and date of last donation.

The age-specific prevalence of active donors peaks at ≈24 years of age and subsequently declines monotonically to zero by age 68. The overall prevalence in the population is 3%, i.e., 2.4 million donors in a population of ≈80 million.

Modeling Transfusion Recipients

The model takes into account that persons may receive >1 transfusion throughout their lifetime, but it does not track the number of transfusions received per person. Persons with ≥1 transfusion continue to be at risk for infection from further transfusions. The age-specific risk of receiving a transfusion was estimated from data for all patients hospitalized at the University Hospital in Essen during March 2003. Of 4,867 patients, 1,343 (27.6%) received ≥1 transfusion. The number of persons receiving a blood transfusion in each 5-year age group was divided by the corresponding number of persons in the general population. The observed rates were fitted with a simple model that assumes initially an exponential decline and subsequently a unimodal peak, which is proportional to the density function of the normal distribution. These age-specific ratios were properly scaled to balance the yearly number of transfusions per capita. To limit the complexity of the model, we did not take into account persons in subgroups, such as those with hemophilia, who obtain blood products from pools of donors. Because for medical reasons these subgroups are excluded from donating blood, they cannot contribute to persistence of the infection.

Independence of Receiving and Donating Blood

The events of receiving a blood transfusion and of donating blood are assumed to be independent of each other. This assumption is supported by the results of a case-control study of potential risk factors for CJD, which was coordinated by the Clinical Surveillance Centre for CJD, Department of Neurology in Göttingen, Germany (*7*). Table 2 shows the joint distribution for the control group of having received and donated blood. According to the Fisher exact test, the p value for the hypothesis of no association is 0.43.

Heterogeneity in the risk of receiving a blood transfusion is modeled by the assumption that only a proportion of the population are at risk, whereas the remaining proportion never receives a transfusion. This assumption was introduced to be consistent with data from the case-control study, in which ≈18% of the population reported having ever received a blood transfusion. Without this assumption, the model would predict that eventually 100% of a cohort would receive a blood transfusion because the average annual risk of receiving a blood transfusion is about 5%, i.e., ≈4 million in a population of 80 million.

Table 2. Joint distribution of transfusion history and blood donation

Received blood	Donated blood, no. observed (no. expected if events are independent)		
	No	Yes	Total no. (%)
No	401 (404)	104 (101)	505 (82)
Yes	93 (90)	19 (22)	112 (18)
Total no. (%)	494 (80)	123 (20)	617 (100)

Modeling Transfusion-associated Death Rates

The transfusion-associated death rate has been described in detail by Wallis et al. (*4*). A good fit to the data assumes that at all ages a certain proportion of transfusion recipients have a higher rate of dying and the remaining proportion has a survival rate that corresponds to that of persons of the same age group in the general population. This age-dependent proportion of transfusion recipients with an increased risk for death is described by a generalized logistic function with a positive value at birth and an asymptote <100% for old age. The transfusion-associated death rate increases linearly with age. The increased death rate appears to be concentrated in the first 2 years after a transfusion. Wallis et al. report that 2,888 patients were observed as long as 7.4 years after transfusions received in June 1994 (*4*). The sex-specific rates were averaged for the simulation model.

Modeling the Infection

Usually the incubation period refers to the time between the infection and disease. In the context of CJD, however, disease can refer to onset, diagnosis, or death. Like Bacchetti, we also focused on death rates (*8–10*). The incubation period is assumed to be gamma distributed with a mean duration of 16 years and a standard deviation of 4 years, which conforms to estimates of Valleron et al. and Ghani et al. (*5,6*). Because of great uncertainty about the length of the incubation time, we also considered a much higher value of 50 years in the absence of the competing risk for death. The coefficient of variation is assumed to be the same, such that the standard deviation is 12.5 years. Because of competing risks, the actual sojourn in the incubation period is 15.3 for an incubation period of 16 years and 34.0 years for an incubation period of 50 years. The proportions of infected persons who would die with disease symptoms are 79% and 37% for the incubation periods of 16 and 50 years, respectively. This means that for an incubation time of 50 years, nearly two thirds would die without disease symptoms. Hereafter we refer to these values of 15 and 50 years as short and long incubation periods.

We distinguish between 2 modes of transmission. Initially, the infection is introduced into the population by the alimentary route. In the United Kingdom the number of infected animals entering the food supply peaked in 1989; most were concentrated within a period of 10 years (*11*), which we take as the assumed period of alimentary infection. After this period, this mode of transmission was interrupted so that further transmissions are possible only through blood transfusions.

A study to detect the presence of abnormal prion protein in appendix and tonsil tissues has suggested a prevalence of 235 infections per million in the United Kingdom (*12*). We arbitrarily assumed the prevalence of infections in Germany to be ≈1 order of magnitude lower, yielding a cumulative incidence of 25 per million, which was the value used for the simulations.

We made 2 contrasting assumptions about the infectivity of blood preparations and evaluated the results of these 2 simulations: each transfusion (100% infectivity) or no blood transfusion (0% infectivity) from an infected donor leads to infection of the recipient. In the model the infection probability (probability of receiving blood from an infected donor) is proportional to the proportion of infected donors among all donors. Thus, we can calculate the number of infections from blood transfusions compared with the number of infections from alimentary transmission alone.

Modeling Donor Exclusion

The model distinguishes between persons with and without transfusion history, termed recipients and nonrecipients; these terms are applied to persons whether they have or have not donated blood. The model allows recipients to be excluded from donating blood. In modeling the exclusion of recipients, we took into account that this measure may be imperfect and that a certain proportion of recipients may not be excluded.

Results

For the parameter estimates obtained from the sources described above, the infection cannot become endemic (Figure 2). If we assume no further spread through blood transfusions after 10 years of infections by the alimentary route, the maximum prevalence reached is ≈1,860 (1,434 for nonrecipients plus 426 for recipients) because some of the infected persons die of other causes during the incubation period. If transmission is assumed to be possible through blood transfusions (100% infectivity), then the maximum prevalence among recipients is increased by ≈78 infections after 4 more years for the short incubation period and by 193 infections after 23 more years for the long incubation period.

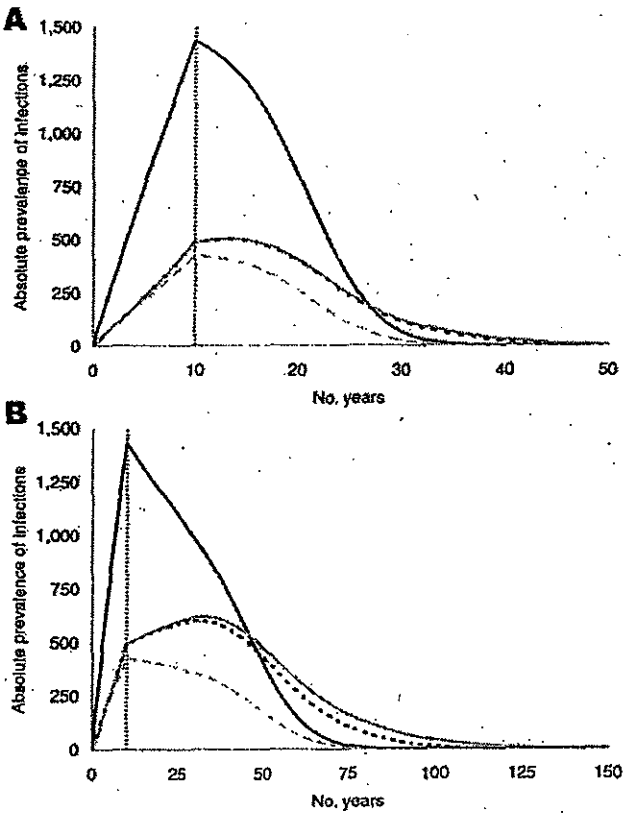

Figure 2. Absolute prevalence of infection for an incubation period of 16 (A) and 50 (B) years, for nonrecipients of blood transfusion (solid, black), recipients under the assumption of no infectivity (dashed, gray), of 100% infectivity without donor exclusion (dotted, black), and 100% infectivity with donor exclusion (solid, gray). The prevalence declines after the alimentary route of transmission is interrupted, i.e., after 10 years. Prevalence differs only slightly if the infection probability of a transfusion from an infected donor is increased from 0% to 100%. Donor exclusion produces negligible reductions.

We assumed that donor exclusion is implemented immediately at the beginning of the alimentary infection risk period, which reduced the original number of 2.55 million donors by ≈20% to a value of 2.05 million donors. Because the model does not account for the stock of blood donations, this reduction in the number of donors must be compensated for with an increased rate of donations per donor to satisfy the demand; i.e., the average number of donations would have to increase from 1.6 to 2 per donor per year. Figure 2A shows that donor exclusion has almost no effect when the incubation period is assumed to be 16 years. The absolute prevalence (i.e., the actual number of infected persons) differs at most by 9. For a long incubation, differences are visible (59 persons at most) but small in view of the long time intervals and the size of the total

population (Figure 2B). The reason for these small differences is described below.

The cumulative numbers of deaths from the infection are given in Table 3. The numbers are considerably smaller for the long than for the short incubation period because a long incubation period implies more deaths from other causes. The numbers are given separately for cases in patients with and without a history of blood transfusion. The route of infection for nonrecipients is alimentary only, whereas the route of infection for recipients is unclear. If we compare the simulations at 100% and 0% infectivity of blood transfusions, we observe 172 and 224 additional cases for the short and the long incubation periods, respectively. These numbers represent 11% of 1,557 and 31% of 725 cases, which would be expected for 0% infectivity for the short and long incubations periods, respectively. For the short incubation period we expect a higher absolute number of alimentary cases but a smaller proportion of transfusion cases than for the long incubation period. The exclusion of donors would prevent only 15 and 50 cases, i.e., ≈15 (0.9%) of 1,729 and 50 (5%) of 949, respectively, at the end of the epidemic. The epidemic lasts for ≈50 or ≈150 years for the short and the long incubation periods, respectively.

The predicted yearly incidence of deaths due to vCJD, separated by transfusion history, is shown in Figure 3. The yearly peak incidence of total deaths would be 128 and 29 for the short and the long incubation periods at 23 and 51 years after the beginning of the epidemic, respectively. For 0% infectivity the peak incidence would be only 5 and 3 cases less for the short and long incubation periods, respectively, which implies that the exclusion of donors with a transfusion history does not effectively prevent infection.

Figure 4 shows the predicted yearly incidence of deaths according to the route of infection. The time lags between the peaks of deaths due to alimentary infection and due to transfusion clearly differ and are 9 and 20 years for short and long incubation periods, respectively.

Finally, we considered the absolute prevalence of infected donors according to their history of blood transfusion (Figure 5). Most infected donors do not have a transfusion history, which explains the negligible effect of a policy excluding transfusion recipients from donation.

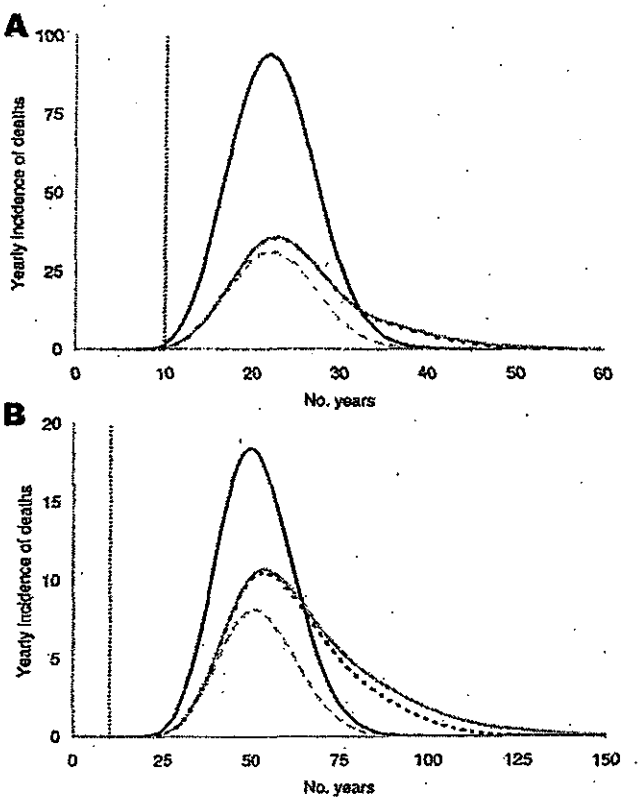

Figure 3. The yearly incidence of deaths for an incubation period of 16 (A) and 50 (B) years. The black curves show nonrecipients of blood transfusion who were infected only by the alimentary route. These curves are independent of the infection probability and the rate of donor exclusion. The lower 3 curves represent the deaths of recipients originating from 0% infectivity of blood transfusions (dashed gray), 100% infectivity without donor exclusion (solid gray), and 100% infectivity of blood transfusions with donor exclusion (dotted black, almost indistinguishable from solid gray line in A). The differences between the solid and dashed gray curves represent the cases due to blood transfusion.

To determine whether the same model could also predict transition into a positive endemic equilibrium of the infection, we made the unrealistic assumptions that the rates of donor recruitment and donor loss are constant between the ages of 18 and 67 and that the rate of receiving a blood transfusion is constant throughout life. Then the model showed an extremely long time (>2,000 years)

Table 3. Cumulative numbers of deaths from variant Creutzfeldt-Jakob disease at the end of the epidemic

Incubation period	Donors excluded	Infectivity (%)	Without transfusion	With transfusion	Total no. cases
Short	No	0	1,167	390	1,557
	No	100	1,167	562	1,729
	Yes	100	1,167	547	1,714
Long	No	0	503	222	725
	No	100	503	446	949
	Yes	100	503	396	899

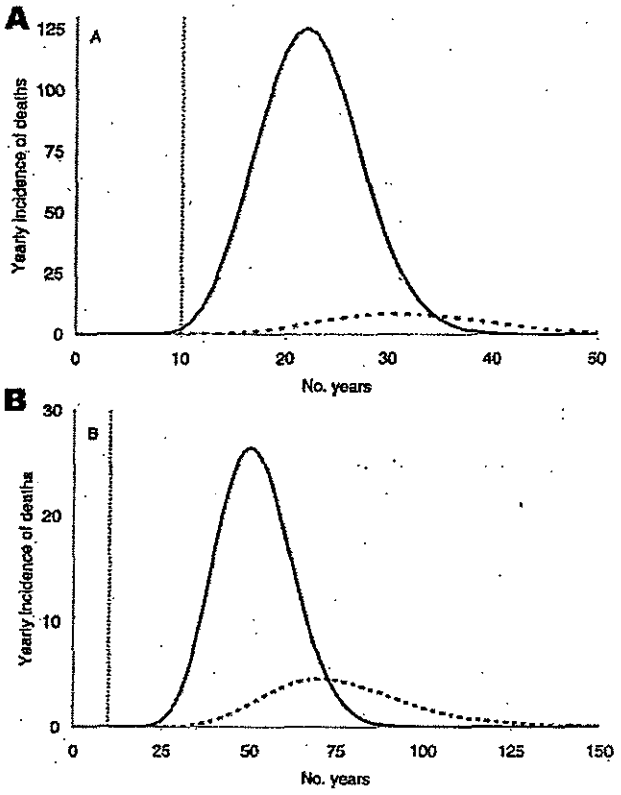

Figure 4. Yearly incidence of deaths caused by alimentary transmission (solid line) and by blood transfusion (dashed line).The 2 peaks differ by 9 and 20 years, depending on the incubation period: 16 (A) and 50 (B) years, respectively.

before positive equilibrium would be reached (results not shown).

Discussion

Our model is the first attempt to describe in a realistic way the transmission of infections through blood transfusions. In 1994, Velasco-Hernández proposed a model for the spread of Chagas disease by vectors and blood transfusion (*13*). His model was used by Roberts and Heesterbeek to introduce their new concept to estimate the effort to eradicate an infectious disease (*14*). Huang and Villasana included transmission through blood transfusion in an AIDS model (*15*). All these models have in common what Inaba and Sekine state about their extension of Velasco-Hernández's Chagas model: "...here we assume that blood donors are randomly chosen from the total population, and so there is no screening and the recipients of blood donations are donating blood themselves at the same rate as anybody else. This is an unrealistic assumption, but we will use it." (*16*). These models implicitly describe transmission through blood transfusion exactly like person-to-person transmission by droplet infections.

The key innovation in our model is the simultaneous incorporation of 6 functions that all depend explicitly on the age of a person: 1) natural death rate, 2) rate of receiving a blood transfusion, 3) rates of donor recruitment, 4) donor loss, 5) death rate associated with transfusions, and 6) proportion of transfusion recipients at increased risk for death. The age-dependent effects of these processes cannot be ignored. Peak ages of donor activity (≈22 years) and of receiving a blood transfusion (≈70 years) are quite distinct and ≈50 years apart. This age pattern does not favor the spread of infection by blood transfusion. Another factor that acts against the infection becoming endemic is the transfusion-associated death rate. The good quality of the follow-up data of nearly 3,000 patients helped to incorporate realistic assumptions about the survival probabilities of transfusion recipients (*4*). The only data available about the joint distribution of blood donor activity and history of a blood transfusion was the CJD case-control study performed in Göttingen, Germany (*7*).

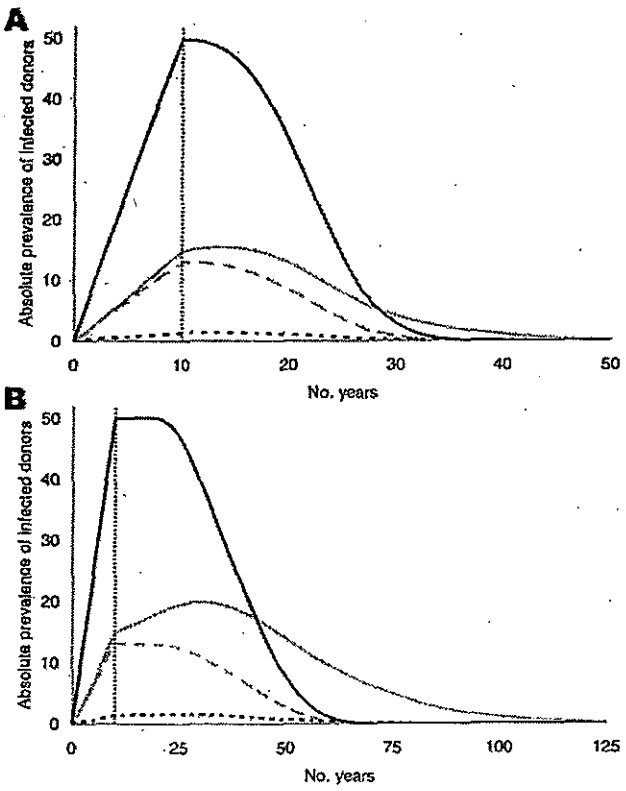

Figure 5. Absolute prevalence of infected donors for an incubation period of 16 (A) and 50 (B) years. The solid black curves show the infected donors without transfusion history. These curves are identical for 0% and 100% infectivity and are independent of donor exclusion. The gray curves show infected donors with transfusion history for 100% (solid) and 0% (dashed) infectivity, respectively, without donor exclusion. The dotted black curves show the effect of donor exclusion starting at the beginning of alimentary risk. Most infected donors have no transfusion history and cannot, therefore, be excluded from blood donation.

The length of the incubation period plays a major role in transmission dynamics and hence was subject to a sensitivity analysis. The model does not account for possible changes of infectivity during the incubation period. The model represents a worst-case scenario because it assumes 100% infectivity throughout the period of infection. Even under this extreme assumption, donor exclusion can prevent only 0.9% (or 5%) of the expected deaths, assuming the incubation period has a mean duration of 16 (or 50) years. The main explanation for this surprising result is that most infected donors have been infected by the alimentary route and never received any blood transfusion and, therefore, are not eligible for donor exclusion.

The present simulations have arbitrarily assumed a cumulative incidence of alimentary infection, about 25 per million (2,000 per 80 million). With pessimistic assumptions, the model predicts either 19.5 deaths per million for the short incubation period or 9 deaths per million for the long incubation period in the absence of spread through blood transfusion. This corresponds to at least 9 (36%) of 25 deaths attributable to the infection, which is ≈2 orders of magnitude higher than expected for vCJD in the United Kingdom. As of July 2006, the number of vCJD cases in the United Kingdom was 160. If we assume that the total number of cases will be 200, then our assumption corresponds to about 3.3 cases per million. Thus, at most, 1.4% of infected persons would die from the infection (unless a second wave of vCJD cases with a long incubation period occurs). According to our model, 0.9% of the deaths could be prevented by donor exclusion under the assumption of the short incubation period. In absolute numbers this would be ≈2 cases.

In France, the total number of vCJD cases recorded through July 2006 is 18. Even under the assumption that this number represents only 35% of the total number of cases (*17*), the absolute expected number of prevented cases would be <1. In 1998, France decided to exclude donors with a transfusion history, primarily to reduce the spread of viruses. The present model could be modified to assess the effectiveness of excluding donors with transfusion history for preventing emerging infections with different modes of transmission and additional epidemiologic states, e.g., latent or immune.

Our worst-case scenario assumptions of the epidemiology might seem similar to the situation in the United Kingdom. In Germany, no case of vCJD has been reported, which indicates that the expected number of cases in Germany is at least 2 orders of magnitude less than that in the United Kingdom. This latter aspect was considered in the interpretation of our model by a working group commissioned by the German Federal Minister of Health, which recommended in April 2006 that persons with a transfusion history not be excluded from donating blood (*18*). Our analysis enables different countries to perform their own risk assessment and choose a strategy according to the absolute number of cases observed or expected.

The German CJD Surveillance study was supported by a grant from the German Ministry of Health (Az 325-4471-02/15 to Inga Zerr and H. A. Kretzschmar). Helpful discussions about previous versions of the model took place with the Working Group Overall Blood Supply Strategy with regard to vCJD, Germany (Chairman R. Seitz).

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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	総合機構処理欄
一般的名称	－		研究報告の公表状況	http://www.fda.gov/cber/blood/fxivcjdqa.htm	公表国：米国	
販売名（企業名）	－					

研究報告の概要

変異型クロイツフェルトヤコブ病（vCJD）が血漿由来の血液凝固第 XI 因子製剤（pdFXI）投与により患者に伝播するリスクが問題となっている。米国では 1989 年から 2000 年にかけて約 50 名の患者に英国供血者血漿由来の pdFXI が投与された。
米国公衆衛生局（PHS）は、vCJD のリスクは小さいものだと考えている。リスク評価にコンピュータモデルを使用したが、多くの未知の要因があるため、正確なリスク評価は行えない。供血時に英国供血者が気付かずに vCJD を保持していた可能性があるため、この pdFXI 製剤を投与された患者には有意なリスクがあるかもしれない。
これまでのところ、英国供血者からの血漿分画製剤を長期にわたり投与された患者も含め、血友病患者や FXI 欠乏症などの血液凝固異常患者に vCJD が発症したという事例は世界的にも知られていない。
2003 年 12 月から 2007 年 4 月までの間に赤血球輸血を通じて vCJD 因子が伝播したとされる 4 例はすべて英国での報告であり、いずれも pdFXI などの血漿分画製剤は関与していなかった。
今のところ、健康な供血者や受血者の vCJD を検出するための検査はない。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
pdFXI の vCJD 伝播リスクは非常に小さいと考えられるとの情報である。 本情報にもあるとおり、現時点まで血漿分画製剤からの vCJD 伝播の報告はなく、血漿分画製剤の製造工程でプリオンが除去できるとの情報もある。 なお、本報告で問題とされている英国供血者血漿は弊社では使用していない。	今後とも vCJD に関する安全性情報等に留意していく。

Potential Variant Creutzfeldt-Jakob Disease (vCJD) Risk

From Investigational Factor Eleven (FXI) From Donors In The United Kingdom

Summary Information

Key Points:

- In recent years, questions have been raised concerning the risk from variant Creutzfeldt-Jakob disease (vCJD), a rare but fatal brain infection, in patients who received plasma-derived investigational Factor Eleven (pdFXI) made from plasma obtained in the United Kingdom (UK) where vCJD has occurred.
- Approximately 50 individuals in the US, between 1989 and 2000, received pdFXI made using plasma from donors in the UK. This product was used to prevent or treat bleeding due to a rare problem, a deficiency of FXI.
- The US Public Health Service (PHS) believes that the risk of vCJD is likely to be small based on a number of considerations. We used a computer model to help determine the risk but we recognize that many unknowns prevent us from accurately determining the risk. The model raised the possibility that those who received this pdFXI product could potentially be at significant risk due to the possibility that a UK blood donor unknowingly carried vCJD at the time of donation. However, we believe the risk is small based on additional considerations. ***To date we are not aware of any cases of vCJD having been reported worldwide in patients with hemophilia or other blood clotting disorders, including pdFXI deficiency, who have received large amounts of plasma-derived products manufactured from UK plasma. This includes patients who received these products over a long period of time.***
- Contacting a specialist in bleeding disorders, e.g. a healthcare provider specializing in hemophilia, and/or a Hemophilia Treatment Center is a good way to learn about any new information as it becomes available.

Additional Information:

- Between December 2003 and April 2007, there have been four reports of people, all in the UK, who probably acquired the vCJD agent through red blood cell transfusions. This has increased concern about the potential transmission of vCJD by blood products, particularly those made from UK blood donors. ***None of the reported cases involved any plasma-derived product, including pdFXI.***
- However, because of the finding that red blood cells can transmit vCJD, FDA used a computer model to conduct a risk assessment to try to estimate the possible risk that might occur from the UK investigational pdFXI.
- The actual risk of acquiring vCJD is unknown and is likely to be small. Because so much is unknown about vCJD and its prevalence, the risk assessment performed by FDA has a lot of uncertainty, making it impossible to precisely estimate the risk of vCJD in general, or the actual risk to individual FXI deficient patients. There is no test yet available to detect vCJD in healthy donors or recipients. The US Public Health Service believes the risk of vCJD is likely to be small. There have been no reports of vCJD in patients using any plasma-derived blood product in the UK or anywhere else in the world.
- At this time, PHS does not believe there is a need for UK pdFXI recipients to inform their surgeons or dentists about the recipient's potential exposure to vCJD. Also, there is no recommendation for surgeons and dentists to take any special precautions based on such potential exposures. This belief is based on the very large degree of uncertainty in the FDA risk assessment and the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK, where risk is considered greatest, or anywhere else in the world. Also, relatively few patients were exposed to the pdFXI product in the US compared to the number of recipients of plasma-derived clotting factors, of which pdFXI is only one of many, in the UK.
- vCJD originally came from a disease in cattle called "mad cow disease" or BSE (bovine spongiform encephalopathy). Transmission of the BSE agent to humans, leading to vCJD, is believed to occur primarily from eating beef and beef products contaminated with the BSE agent. Both BSE and vCJD are invariably fatal brain diseases with incubation periods typically measured in years.
- From 1995 through April 2007, 202 individuals with vCJD were reported worldwide, with 165 in the United Kingdom (UK), and three in the United States. Two of the individuals in the United States had lived in the UK from 1980-1996 during a key exposure period to the BSE agent. The third US individual with vCJD most likely acquired the infection in Saudi Arabia. The reported incidence of vCJD in the UK, based on disease onset, peaked in 1999 and has been declining thereafter. In the UK, where most cases of vCJD have occurred, the current risk of acquiring vCJD from eating beef and beef products appears to be negligible.
- More information about vCJD is available on these government websites:
  - FDA: Potential Risk of Variant Creutzfeldt-Jakob Disease (vCJD) From Plasma-Derived Products
  - Centers for Disease Control and Prevention: vCJD (Variant Creutzfeldt-Jakob Disease)
  - US Department of Agriculture
- Information also may be obtained from these non-government sources:
  - Committee of Ten Thousand
  - Hemophilia Federation of America
  - National Hemophilia Foundation and/or HANDI
  - World Federation of Hemophilia

Updated: May 30, 2007

Questions and Answers

Variant Cruetzfeldt-Jakob Disease (vCJD) and Factor XI (pdFXI)

Index

Q. What is vCJD and how is it spread?

A. Variant Creutzfeldt-Jakob disease, or vCJD, is a very rare, fatal disease that can infect a person for many years before making them sick by destroying brain cells. Eating beef and beef products contaminated with the infectious agent of bovine spongiform encephalopathy (BSE) is the main cause of vCJD.

Most cases of vCJD have occurred in the United Kingdom (UK). Individuals in the UK are at a greater risk for this rare disease than are individuals elsewhere because of the previous higher risk of potential exposure to contaminated beef in the UK diet. From 1995 through April 2007 there have been 202 individuals with vCJD reported worldwide, 165 of them in the UK. In the United States (US), there have been three reported cases of vCJD. Two of these individuals had lived in the UK during 1980-1996, a key exposure period to the BSE agent. The third US individual with vCJD probably acquired the infection in Saudi Arabia.

The reported incidence of vCJD in the UK, based on disease onset, peaked in 1999 and declined thereafter. In the UK, where most cases of vCJD have occurred, the current risk of acquiring vCJD from eating beef and beef products appears to be negligible.

Only three cases of BSE have been found in US cattle, and safeguards are in place to help prevent infected beef products from entering our food supply. These safeguards include restricting importation of cattle and beef products from almost all countries with BSE, a surveillance program to detect BSE in the US, prohibiting the use of high-risk animal-derived proteins in cattle feed, prohibiting meat from sick cattle to be used for human consumption, and requiring the removal of high-risk materials from carcasses of cattle over a certain age.

While vCJD is primarily due to eating infected beef and beef products, four people in the UK became infected with the vCJD agent after receiving red blood cells from three donors who later developed vCJD. Three of the red blood cell recipients developed typical vCJD and died from the disease. A fourth died of an unrelated illness but had evidence of infection. To date, there have been no reports of vCJD transmission by close personal contact (such as being in the same room with someone who has vCJD, hugging, kissing, or having sexual relations).

Q. How does vCJD differ from Creutzfeldt-Jakob disease (CJD)?

A. Both vCJD and CJD cause progressive degeneration of the brain leading to death. However, the variant form—never seen before 1994—usually affects persons much younger than other forms of CJD. Unlike CJD, vCJD has been acquired by food exposure and transmitted by blood transfusion. vCJD also has somewhat different clinical symptoms, a longer survival after onset of illness (the majority of illnesses lasting more than one year), and produces a characteristic abnormality in brain tissue called "florid plaques" rarely if ever seen in the other forms.

Q. Is it known that pdFXI can transmit vCJD?

A. No. However, pdFXI is made from plasma. Plasma is the liquid part of blood remaining after the cells are removed. Animal studies show that if blood carries the vCJD agent, so can the unprocessed plasma.

Manufacturing steps used in making pdFXI have been shown to help remove infectious agents, including agents similar to that causing vCJD. The manufacturing steps may reduce or eliminate most risk even if a vCJD-infected donor contributed plasma.

Q. What is the likelihood that a patient who received pdFXI could have become infected with vCJD?

A. The US PHS believes the risk of developing vCJD infection from pdFXI is likely to be small. Many unknowns prevent us from accurately determining the risk using a computer model, and we believe the risk is likely to be smaller than the modeling predicts. However, we do not know this with certainty. Right now, there is no test available to detect vCJD in blood donors or recipients. There is no way of knowing whether a person is infected if

they do not show symptoms of the disease.

At this time FDA, CDC, and NIH are not aware of any cases of vCJD having been reported worldwide in patients receiving plasma-derived clotting factors, including pdFXI. This includes patients who have received, over a long period of time, large amounts of clotting factor products manufactured from plasma donations from the UK, where the risk of vCJD is highest.

Q. Why did FDA do a vCJD risk assessment for pdFXI made from UK plasma?

A. We conducted a risk assessment on pdFXI because it was made from plasma obtained from donors in the UK. The UK population, including UK plasma donors, is at a considerably higher risk for vCJD than the US population due to eating food potentially contaminated with the BSE agent, although the estimates of risk vary widely. We believe that pdFXI is the only plasma product used in the US that was manufactured from UK donor plasma collected during the BSE epidemic. Note, however, that plasma pools used to manufacture the pdFXI product infused in the US did not contain donations from individuals known to have developed vCJD (that is, there were no known "implicated" lots).

Q. Why is FDA informing patients, healthcare providers, and the public about vCJD and pdFXI now?

A. The FDA has recently completed its risk assessment, and we think it is important that a person who received pdFXI be aware of the results of the risk assessment and have an opportunity to discuss any questions with a suitable health care provider.

The first case of probable vCJD infection transmitted by transfusion of red blood cells in the UK was reported in December 2003 and the second case in July 2004. These events prompted UK authorities in 2004, to communicate the potential risk of vCJD to recipients of clotting factors and some other plasma derived products. FDA initiated its risk assessment for pdFXI in 2004, and presented a draft to the Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC) in February 2005 (draft risk assessment, meeting transcript and slides). Since then FDA, with scientific advice from the TSEAC in October 2005, and other experts, has further refined the risk assessment and risk communication materials. Results of this extensive analysis are now available.

FDA, CDC, NIH, and the Office of Public Health and Science (OPHS) of the US Department of Health and Human Services, with advice from patient advocacy groups and communication experts, have now developed key message points and communication materials to accurately convey the possible risk to patients, health care providers, and others who may have an interest.

Q. Should patients inform their primary health care providers about a possible vCJD exposure from UK pdFXI?

A. Advising your primary health care provider (e.g., a family physician, internist, blood disease specialist, etc.) about your history of having received the pdFXI product might be beneficial in that your provider can keep you informed about new information as it becomes available, interpret its significance, and advise you about further appropriate actions in the future. However, sharing your personal health information is your choice.

Q. Do patients who received UK pdFXI need to do anything special when seeking dental or surgical care?

A. At this time, the US PHS does not believe that UK pdFXI recipients need to inform their surgeons or dentists about the potential exposure to vCJD. Also, the US PHS does not recommend that surgeons and dentists take any special precautions with patients who had such potential exposures. This belief is based on the very large degree of uncertainty in the FDA risk assessment, and the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK, where risk is considered greatest, or anywhere else in the world. Also, there were relatively few patients exposed to the pdFXI product in the US compared to the large number of recipients of plasma-derived clotting factors, of which pdFXI is only one of many, in the UK.

In the UK, public health authorities notified recipients of plasma-derived products, such as pdFXI, that they may have an increased risk of vCJD in addition to their risk from eating potentially contaminated UK beef products. The UK health authorities asked patients to inform their surgeons and dentists about their potential exposure as a public health precaution intended to prevent possible secondary spread of the disease from dental and surgical instruments. The US PHS, including the FDA, CDC, and NIH, does not believe that such notifications are necessary in the US. This belief is based on the very large degree of uncertainty in the FDA risk assessment, and on the lack of known cases of vCJD transmitted by plasma-derived clotting factor products in the UK or anywhere else in the world. Given this information, the PHS believes that there is no need to alter the standard current practices.

- PHS agencies will continue to monitor and reevaluate the situation as new information becomes available.

Q. What can recipients of pdFXI do with this information?

A. While no new actions are recommended now, you can stay informed by keeping in contact with your primary physician and/or a specialist in bleeding disorders, such as a hemophilia specialist at a Hemophilia Treatment Center. Such contact will help you to learn about any new scientific advances in this field such as testing and diagnosis, and also to monitor your general health.

Q. What are Hemophilia Treatment Centers, and where can I find out about them?

A. Hemophilia Treatment Centers (HTC) are a network of federally funded comprehensive care clinics that promote the management, treatment, and prevention of complications experienced by persons with hemophilia

and other hereditary bleeding disorders.

You can find information about HTC's at:

1. CDC informational posting, containing information about the kinds of services provided by federally funded HTC's
2. CDC's directory of federally-funded HTC's
3. Regional HTC websites are also a good place for information

Q. Where can I find more information about vCJD and pdFXI?

A. You can find additional information at:

FDA

1. FDA informational posting, containing current pdFVIII risk assessment, fact sheet, and briefing materials
2. Blood Products Advisory Committee meeting – summary of recent TSEAC meeting and statement about pdFXI from the UK, on October 21, 2006
3. TSEAC meeting with discussion of first pdFXI draft risk assessment, on February 8, 2005, and discussion of UK risk communication for plasma derivatives
4. TSEAC Meeting with further discussion of the FDA risk assessment model, October 31, 2005
5. TSEAC Meeting with update on pdFXI risk assessment, September 18, 2006

CDC: vCJD (Variant Creutzfeldt-Jakob Disease)

Regional HTC websites

US Department of Agriculture: Bovine Spongiform Encephalopathy

Patient Organizations:

Committee of Ten Thousand
Hemophilia Federation of America
National Hemophilia Foundation and/or HANDI
World Federation of Hemophilia

Questions to FDA may be addressed through the Office of Communication, Training, and Manufacturers Assistance (OCTMA), at 1-800-835-4709, or octma@cber.fda.gov.

Updated: May 30, 2007

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	Transfusion clinique et biologique-journal de la Societe francaise de transfusion sanguine (France) Nov 2006, 13 (5) p320-8.	公表国 仏国	
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
血漿分画製剤は、原材料がヒト由来であるため、変異型クロイツフェルト・ヤコブ病（vCJD）を引き起こすプリオンを伝播する可能性があるが、血漿分画製剤によってプリオンが伝播したという報告はない。 様々な国で実施されている対策（BSE 発生国での滞在歴や輸血または組織移植を受けたことがある献血者の保留、白血球除去など）や、いくつかの製造工程が、血漿分画製剤によるプリオン伝播防止に貢献している可能性がある。 脳由来の感染因子をスパイクする多数の実験的感染性試験により、いくつかの分画ステップ、特にエタノール分画、デプス・フィルトレーション（深層濾過）、イオン交換クロマトグラフィーは、プリオンを数 log 除去できることが実証された。更に、凝固因子や免疫グロブリン製剤からウイルスを除去するために使用されている 75 nm 以下の多層膜を使ったナノろ過は、サイズ的排除とトラッピングメカニズムによって、スパイクしたプリオンを 3～5 log 以上除去することができる。 これらのことから、血漿分画製剤による vCJD の伝染はほとんどないと思われるが、ヒト血液中における感染因子の生化学的性質についてはまだ分からないところがあるため、注意を怠るべきではない。	**重要な基本的注意** 現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的な vCJD 等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

報告企業の意見	今後の対応
血漿分画製剤からの vCJD 感染リスクに関する情報で、現時点まで血漿分画製剤からの vCJD 伝播の報告はないこと、血漿分画製剤の製造工程でプリオンが除去できるとの情報である。	今後とも vCJD に関する安全性情報等に留意していく。

Available online at www.sciencedirect.com
ScienceDirect
Transfusion Clinique et Biologique 13 (2006) 320–328
TRANSFUSION CLINIQUE ET BIOLOGIQUE
http://france.elsevier.com/direct/TRACLI/

Original article

Current strategies to prevent transmission of prions by human plasma derivatives

Mesures actuelles de prévention du risque de transmission de prions par les médicaments dérivés du plasma humain

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Abstract

Protein products prepared from pooled human plasma are an essential class of therapeutics used mostly to control bleeding and/or immunological disorders. Because of the human origin of the starting material, there is a risk that these products may possibly transmit prions causing variant Creutzfeldt–Jakob disease (vCJD). No case of transmission of prions by plasma products has been observed. Case-by-case measures implemented in various countries, and several technical factors may contribute, to various degrees, to the prevention of the risk of transmission of prions by plasma products. Those measures include (a) the epidemiological surveillance of population in countries with cases of vCJD and/or bovine spongiform encephalopathies (BSE), (b) the deferral of blood donors who traveled or resided, for specific periods of time, to countries with BSE, or who received transfusion or tissue transplant, (c) the removal of leucocytes in plasma used for fractionation, and, last but not least, (d) the removal of the prion agents during the complex industrial fractionation process used to prepare plasma products. Numerous experimental infectivity studies, involving the spiking of brain-derived infectious materials, have demonstrated that several fractionation steps, in particular ethanol fractionation, depth filtration, and chromatography, can remove several logs of prions. Removal is explained by the distinct hydrophobic and aggregative properties of the prion proteins. In addition, nanofiltration using multi-layer membranes of 75 nm or smaller, which is commonly used for removing viruses from coagulation factors and immunoglobulins products, can remove more than 3–5 logs of spiked prions, presumably by size-exclusion and trapping mechanisms. Therefore, the risk of transmission of vCJD by human plasma products appears remote, but caution should prevail since the biochemical nature of the infectious agent in human blood is still unknown.

Résumé

Les médicaments dérivés du plasma humain occupent une place thérapeutique essentielle en particulier dans le traitement de troubles hémorragiques ou immunologiques. Par l'origine humaine du plasma, ces produits sont une source possible d'infection par les agents transmissibles non conventionnels (ATNC ou prions), dont celui induisant la variante de la maladie de Creutzfeldt–Jakob (vMCJ). On n'a recensé toutefois à ce jour aucun cas de transmission de vMCJ par les produits plasmatiques industriels. Diverses mesures de précaution, mises en place au cas par cas dans différents pays, et des facteurs techniques paraissent prévenir les possibilités de transmission de ces agents infectieux par les médicaments dérivés du sang. Ils comprennent : (a) le contrôle épidémiologique de la population dans les pays où des cas de vMCJ et/ou d'encéphalopathie bovine spongiforme (EBS) ont été identifiés ; (b) l'exclusion des candidats donneurs de sang ayant voyagé ou séjourné pour une certaine période de temps dans des pays touchés par l'EBS, ou ayant été transfusés ou transplantés ; (c) la limitation du contenu du plasma en leucocytes ; et (d) l'élimination de la protéine prion pathologique au décours des étapes de fractionnement. De nombreuses études expérimentales, reposant le plus souvent sur des épreuves de surcharge par extraits de cerveaux d'animaux infectés par une souche d'ATNC, concourent à établir que diverses étapes de fractionnement, dont les précipitations en présence d'éthanol, les filtrations en profondeur, et les chromatographies, contribuent à une élimination importante des prions. Celle-ci paraît s'expliquer par les caractéristiques d'hydrophobicité et de tendance de l'agent infectieux à

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doi:10.1016/j.tracli.2006.11.001

s'agréger. Par ailleurs, les étapes de nanofiltration sur des membranes multicouches d'une porosité de 75 nm ou moins, utilisées pour la sécurisation virale des facteurs de coagulation ou des immunoglobulines, retiennent, vraisemblablement par des mécanismes d'exclusion stérique et de piégeage, plus de trois à cinq logs de prions. Au regard de ces données expérimentales, le risque de transmission de vMCJ par les produits plasmatiques paraît très mince, mais la portée réelle de ces études reste incomplète tant que la nature de l'agent infectieux présent dans le sang ne sera pas pleinement élucidée.

Keywords: Prions; Plasma products; Fractionation; PrP^{TSE}; Safety; vCJD

Mots clés : Prions ; Agents transmissibles non conventionnels ; Produits plasmatiques ; Fractionnement ; PrP^{TSE} ; Sécurité ; vCJD

1. Introduction

Human plasma is used for the manufacture of fractionated products called plasma products, plasma derivatives, or plasma-derived medicinal products. These products have no substitutes to treat various life-threatening congenital or acquired bleeding, thrombotic disorders, immunological deficiencies, tissues enzymatic degradations, and/or trauma. Plasma products are purified from pools of thousand liters of plasma. Production methods involve sophisticated purification procedures based on a series of precipitation, filtration, and chromatographic steps that constitute the plasma fractionation process [1]. As historical perspectives show, various transmissible infectious agents, most notably viruses, can contaminate human blood and be transmitted by industrial plasma products. It is now believed that the variant form of Creutzfeldt–Jakob disease (vCJD), a human disease thought to be primarily associated to the ingestion of food contaminated by the bovine spongiform encephalopathy (BSE) agent, may also be transmitted by transfusion of blood components [2]. Three human cases of transfusion transmitted prion infections (only two of which were symptomatic of vCJD) have been identified and ascribed to the infusion of non-leucoreduced red blood cell concentrates [3]. The third, pre- or sub-clinical, case was in an individual who was Met/Val heterozygous at codon 129 of the prion protein gene, suggesting that individuals with a genotypes other than Met–Met homozygous at residue 129 may also be infected [3,4].

As it is now apparent that vCJD can be transmitted by transfusion, concerns about the safety of pooled plasma derivatives have grow. A UK retrospective study on tissue samples suggests that more people than initially thought may be incubating the disease [5,6]. There is, so far, no evidence that vCJD has been transmitted by plasma derivatives. However, the possibility of long incubation period of the disease does not allow to draw definite conclusion on the absence of risks, and therefore preventative measures have been put in place in several countries. The purpose of this paper is to present the current strategies that are believed to restrict the risk of transmission of prions by industrial plasma products.

2. Preventative measures for the collection of plasma for fractionation

The occurrence of human-to-human transmission of vCJD by blood transfusion has alerted health regulatory authorities on the importance of implementing a set of precautionary measures. Table 1 summarizes the current preventative measures that are in place against the risk of transmission of prions by plasma products. The approach largely follows, when possible, the one that has been developed successfully over the last 20 years to avoid the transmission of viruses. The strategy needed to prevent transmission of viruses requires (a) an accurate information on the structure of the infectious agents and their mode of transmission, (b) the epidemiological surveillance of the population, (c) the accurate screening of the donors, (d) the testing of the donations by sensitive and relevant assays, (e) the implementation of validated inactivation or removal procedures during product manufacturing, and (f) the application of good manufacturing practices (GMP) at all stages of the production chain [7,8]. As indicated in this publication, not all of these measures can be used to date to reduce the risks of transmission of prions by industrial plasma products.

2.1. Biophysical characteristics and resistance of PrP^{TSE}

The physico-chemical properties of human blood-associated transmissible spongiform encephalopathy (TSE) agent are still unknown. The infectious agent is thought to be a misfolded, abnormal, prion protein, globally now referred to as PrP^{TSE} because of increasing complexity in the terminology for various forms of the prion protein [4]. Pathological PrP^{TSE} represents aberrantly folded isoforms of a normal cellular prion protein (PrP^{c}) whose physiological function is still largely unknown. PrP^{c} is a glycosyl phosphatidyl inositol-linked glycoprotein composed of approximately 256 amino acids, that undergoes facultative N-linked glycosylation at two sites [9, 10]. PrP^{TSE} present β-sheet structure that tends to aggregate and which, in vitro, makes it insoluble in detergent solutions, resistant to enzymatic degradation, and prone to adhere to surfaces [11]. Recent experimental studies in scrapie-infected (263K strain) hamster brain suggest that prion particles have a size and molecular weight range of 5–90 nm, and 155–15,220 × 10^3 KDa, respectively. Infectivity of this prion strain is highest in oligomers with apparent radii of 17–27 nm and a molecular weight of 300–600 kDa, and is apparently absent, or less, in large fibrils and in oligomers of ≤ 5 PrP molecules [12]. Non-fibrillar particles, with a mass equivalent to 14–28 PrP molecules, may be the most efficient initiators of TSE disease [12].

Table 1
Points to consider and measures to prevent the transmission of viruses and prions by plasma-derived medicinal products

Points to consider/measures	HIV, HBV, HCV	HAV, B19	Prion agent
Structural characteristics of the infectious agent	Well characterized (enveloped, size, shape, genome, resistance)	Well characterized (non-enveloped, size, shape, genome, resistance)	Unknown for the infectious agent in plasma
Infectious dose in human plasma	Known (can reach several logs)	Known (can reach several logs)	Limited information (estimated to be low; 2–30 infectious doses per ml)
Epidemiological information on prevalence, risk factors, and transmission modes	Well established	Well established	Unknown
Specific donor exclusion criteria	Yes	No	Yes[a]
Testing of individual donations for markers of the pathogenic agent	Anti-HIV 1 and 2 Ab HCV Ab HBsAg	No	No (tests in development)
Leucoreduction	No	No	About 50% reduction in infectivity [27]
Plasma pool testing	Yes (e.g. nucleic acid test)	Yes (e.g. nucleic acid test)	No
Dedicated inactivation steps	Yes (e.g. SD treatment, pasteurization, low pH, caprylic acid, dry heat)	Yes/No	No
Unspecific removal steps	Yes (e.g precipitation, chromatography)[b]	Yes (e.g precipitation, chromatography)[b]	Yes? (e.g. precipitation, depth filtration, chromatography)[c]
Dedicated robust removal steps	Yes (nanofiltration)	Yes (nanofiltration)	Yes (nanofiltration)?[c]
Final product testing to control markers of infectious risks	No[d]	No[d]	No

[a] In some countries exclusion criteria includes travel to countries with BSE, and previous transfusion or transplantation (see text for details).
[b] Often not regarded as a robust removal step by most regulatory agencies due to the difficulty in proving consistent viral removal.
[c] This assumes that PrP^{TSE} in plasma would behave the same as PrP^{TSE} from brain homogenates, which is still unknown.
[d] Tests to detect the presence of viral markers in final products have not been validated and do not guarantee product safety.

Brain-associated forms of PrP^{TSE} are resistant to the viral inactivation procedures used during the manufacture of human plasma products, such as solvent-detergent (SD), heat treatments, and low pH [1,13]. The methods known to inactivate PrP^{TSE} (such as oxidation, treatment with strong base, chaotropic agents, extreme heat, strong sodium hypochlorite solutions or hot solutions of sodium hydroxide [14]) would denature plasma proteins and therefore cannot be used in plasma fractionation.

2.2. Prion infectious dose in plasma

There is only limited information on the infectious dose of prion protein in human blood. Estimates based on animal models suggest that prion protein infectivity in blood is low, possibly comprised between 2 and 30 infectious dose per ml during both the incubation and symptomatic stages of disease [15, 16]. Half of the infectivity appears associated with plasma [17]. Possibility of abnormal prion protein transmission by transfusion in humans corroborated earlier experimental evidence that the blood of infected rodents, cows, and sheep may transmit infectivity [18] in both the incubation period and clinical phase [4]. Experimental evidence of blood transfusion transmission in deers with chronic wasting disease has also been reported recently [19]. At the early stages of the incubation period, blood-associated PrP(Sc) may originate from peripheral replication of prions, whereas during the symptomatic phase, it may leak from the brain [20]. Therefore, it can not be excluded that the risk of infectivity varies during the various phases of the disease.

2.3. Epidemiology surveillance

Human exposures to the BSE agent appear primarily linked to (a) the prevalence of BSE in native and imported cattle population and (b) the risk of contamination of local and imported food by the BSE agent. vCJD appears limited geographically to countries where BSE has been identified but not much is known on BSE agent infectivity and on the minimal oral doses able to transmit vCJD to humans [4]. In addition, prion strains distinct from that causing BSE have recently been isolated from cattle, and found to induce lethal neurological disorders in a transgenic mice model, raising potential additional public health concerns [21]. Proportion of asymptomatic carrier may be higher than initially thought [5,6] but further data must be obtained. Countries are therefore encouraged to establish epidemiological surveillance systems and conduct systematic assessment of possible cases of BSE and vCJD to follow the prevalence and trends of the disease so that appropriate deferral measures can be taken on a timely manner, if needed [4].

2.4. Deferral of "at-risk" donors

Deferral criteria of donors presumed at risk of vCJD have been implemented in some countries following a careful assessment of the risk/benefit ratio, the consideration on the long incubation period of vCJD after oral exposure to BSE contaminated beef, and the impact on the supply of blood and plasma products. The rationale for some deferral measures is based on the mathematical probability of "recycling" vCJD infection via blood transfusion and plasma products, consider-

ing the assumed number of infected individuals in a given country. Several countries defer donors who visited or resided in the UK and other European countries where BSE cases have been found, for a cumulative period of 3 months or more between 1980 and 1996. To prevent secondary spread, previously transfused donors are also deferred in countries like France (since 1998), UK (since 2005), Ireland, the Netherlands, and Switzerland. Canada, Australia, Italy and the US are currently deferring donors previously transfused in a country where BSE or vCJD cases have been identified [4].

2.5. Testing

There is as yet no assay available to detect misfolded prion proteins in human blood, one technical difficulty being linked to the low level of prion protein in blood and the fact that the disease does not induce conventional immune response and has no nucleic acid associated markers. Several assays using different principles are under development [4,22] but so far data have not been reproduced by independent groups. It is not known when validated screening tests will be available for routine use in blood establishments. Possible implementation would raise a number of concerns including how to deal with the potential high number of false-positive results. It is also expected that two validated tests, one used for primary determination, the other as a confirmatory assay, would be required [22].

2.6. Leucoreduction

Limiting the leucocytes number in plasma for fractionation to less than 10^6 per l is currently the practice in countries like France, as part of a set of precautionary measures to reduce the risks of vCJD transmission by plasma products [23]. Such limit in leucocyte content can be met by using leucoreduction filters during blood or plasma preparation [24] or by collecting plasma using specific apheresis procedures that reduce leucocytes [25]. The rationale for limiting leukocyte contamination was based on the belief that lymphocytes play a crucial role in TSE pathogenicity [26]. However, leucofiltration of whole blood from hamsters infected with scrapie 263K (endogenous TSE model) was recently found to remove "only" 42–72% infectivity from plasma [17,22,27]. These findings appear consistent with the low reduction of infectivity found by leucofiltration of blood from mice infected with a mouse-adapted strain of human TSE [28]. In an exogenous model where scrapie 263K was used to spike human blood, filtration with four different whole blood leucoreduction filters did not remove significant PrP^{sc} [29]. However, it is still not known how much prion infectivity is removed when using apheresis procedures that reduce the leucocyte content to a range similar to that of whole blood passed through dedicated leucoreduction filters [25].

2.7. Prion removal filters

Currently, two CE-marked filters are commercially available for the capture of prions from red blood cell concentrates [10,15], and a third one, that combines leucoreduction and prion reduction, is in development [30]. Coagulation factors have been found to bind on some of these filters. It is not known whether prion removal filters dedicated for the filtration of plasma for fractionation (or transfusion) will be developed and licensed. Use of prion reduction filters during the plasma fractionation process, rather than at the stage of plasma preparation, may be a more economical and rational approach.

3. Removal of prions during plasma fractionation

3.1. Plasma fractionation technology

Most plasma products are manufactured by an integrated technology encompassing cryoprecipitation, cold ethanol precipitation, filtration and chromatographic steps to achieve protein separation and polishing. Cryoprecipitation, the first step in the fractionation process, is a thawing of plasma at 2–4 °C that isolates a cold insoluble fraction (cryoprecipitate), used as a source of factor VIII (FVIII), von Willebrand factor (VWF), and fibrinogen, and a supernatant (cryo-poor plasma, or cryosupernatant), which is the starting material of other proteins. The ethanol fractionation process—used for instance in albumin, IgG, alpha 1-antitrypsin separation—comprises precipitation steps at 8–40% ethanol concentrations, under defined conditions of pH, temperature, and osmolality [1]. Precipitates and supernatants are separated by centrifugation or filtration using filter aids and depth filters. Chromatography is used for protein separation and purification from the various intermediates, as well as for removal of the solvents and detergents used in viral inactivation procedures [1,31]. Common chromatographic methods include anion-exchange, cation-exchange, immobilized heparin affinity, and immunoaffinity. To date most albumin and IgG preparations are produced by a process largely based on the ethanol fractionation method, while the manufacture of most coagulation factors, protease inhibitors, and anticoagulants preparations involves chromatography [31,32].

3.2. Experimental prion clearance studies

Studies have been carried out to evaluate the clearance of prions taking place during plasma fractionation. These studies are difficult, time-consuming, and expensive. Important factors in their design include the choice of the TSE strains and of the tissue used for spiking, the type of infectivity assay, and—as for viral validation studies [7,33]—the validity of the scale-down process to mimic the large-scale manufacture conditions. Since spiking agents may have different partitioning properties, process clearance is often evaluated using spikes exhibiting different biophysical properties [34]. TSE strains used in exogenous spiking experiments include (a) hamster-adapted scrapie (strains 263K [35], Sc237 [34] or ME7 [36], (b) murine-adapted BSE, 301 V [37] and (c) strains of human CJD, vCJD, sporadic CJD (sCJD) and GSS [38]. Endogeneous studies have used murine-adapted GSS, Fukuoka-1 strain [39], hamster-adapted scrapie, strain 263K and murine-adapted

BSE strain 301 V [40]. Two approaches are available to assess infectivity. Bioassays, which detect infectivity in rodent models [28,37,39,41], are the current "gold standard", but they have limitations linked to the limited availability of specialized animal facilities, their time-consumption, or the inadaptability for evaluation of process robustness [42]. Immuno-chemical determination of PrP^{TSE} is done either by Western blotting [35,43] or, less frequently, conformation-dependent immunoassay (CDI) [34], after digestion with proteinase-K. Immuno-chemical assays are rapid and relatively cheap, and are useful for an evaluation of clearance. Good correlation between both types of assays have been found [41,44]. Tissue culture infectivity assays (TCIA) may be new promising alternatives to animal assays. Sensitivity is equivalent to animal assay and almost 100 times more than the WB. Protein misfolding cyclic amplification (PMCA) which allows autocatalytic replication of minute quantities of infectious prions, is claimed to provide over 4000-times more sensitivity than the animal bioassay [45] and, if validated, may be of interest for clearance studies in the future.

3.3. Results of experimental clearance studies

3.3.1. Coagulation factors

Coagulation factor concentrates are prepared from plasma intermediates generated early in the fractionation process and could be, in principle, at higher risks of contamination by prions. Significant efforts have, understandably, been made to study TSE infectivity removal capacity of chromatographic purification and nanofiltration steps, which are commonly used to manufacture these products.

3.3.1.1. Chromatography. Table 2 summarizes clearance data obtained during chromatography. Various spikes have been used; assays included in vitro immunochemical methods and animal bioassays. Anion-exchange chromatography on DEAE-Toyopearl 650M, as used in the purification of FVIII and fibrinogen [46], and on DEAE-Sepharose, as used for FIX [47], contributes to a significant removal (typically in the 2 to > 3 logs range) of spiked TSE agents [35,42,48,49]. Upstream SD treatment of the cryoprecipitate extract did not impact prion removal [50]. Immobilized heparin affinity chromatography of FIX [47] removed 1.4 $\log_{10}$ of PrP^{TSE} [35], and S-Sepharose cation-exchanger during thrombin purification removed 2.9 $\log_{10}$ [35], and similar removal has been reported by monoclonal antibody chromatography [42]. The fact that consistent prion clearance factors are found in processes using chromatographic resins of different chemical structures and substitutions, and under different buffer systems, supports the occurrence of non-specific binding of the infectious agent onto the chromatographic support surface. Although prion removal appears reproducible, incomplete understanding of the removal mechanism raises questions, such as how to (a) determine the maximum capacity of chromatographic support to bind TSE agents, (b) ensure efficient sanitizing procedures of recycled gels, and (c) guarantee consistent prion removal over production cycles.

3.3.1.2. Nanofiltration. Nanofiltration is a proven, dedicated method using nm-membranes that are permeable to proteins but retain infectious agents (viruses) by size-exclusion partitioning [51,52]. Accumulating experimental evidence (Table 3) shows consistent removal of substantial doses of TSE agents spiked to plasma fractions through multi-layer filters with porosity of 75 nm or less [42]. The removal capacity of the larger pore-size nanofilters (75 and 35 nm) appears, as expected, somewhat influenced by the physico-chemical characteristics of the plasma fraction [53]. Removal of spiked scrapie agent ME7 by 15 nm nanofilters appears more robust and was not, in experimental studies, influenced by 0.9% sarkosyl [36].

3.3.2. Albumin and IgG

Significant work has evaluated the extent of TSE infectivity removal during the backbone plasma fractionation process steps that generate the various plasma intermediates used to manufacture several products (Table 3).

3.3.2.1. Cryoprecipitation. In initial endogenous experiments that studied the fractionation of murine plasma from animals infected with a human TSE, the infectivity was found to precipitate predominantly into cryoprecipitate (and precipitate I+II+III) [39]. However, in exogenous studies where human blood was spiked with hamster-adapted scrapie 263K, only 0.7% of the initial infectivity was recovered in the cryoprecipi-

Table 2
Extent of TSE agent removal during chromatography of plasma-derived coagulation factors. Adapted from Refs. [41,43]

Processing step evaluated	End-product	Agent	Spike	Assays	Reduction factor
IEC (DEAE-Toyopearl 650M)	FVIII	263K	MF	WB	*1.7* [42]
SD + IEC (DEAE-Toyopearl 650M)	FVIII	263K	MF	WB	*3.1* [35]
SD + IEC (DEAE-Toyopearl 650M)	FVIII	BSE (strain 301V)	MF	Bioassay	*2.7* [48]
IEC (DEAE-Toyopearl 650M)	vWF	263K	MF	WB	*3.9* [42]
IEC (DEAE-Toyopearl 650M)	Fibrinogen	263K	MF	WB	*3.8* [42]
SD+ IEC (DEAE-Toyopearl 650M)	Fibrinogen	263K	MF	WB	*≥ 3.5* [35]
SD+ IEC (DEAE-Toyopearl 650M)	Fibrinogen	BSE (strain 301V)	MF	Bioassay	*2.9* [48]
DEAE-cellulose	Prothrombin complex/FIX	263K	MF	WB	*3.0* [35]
IEC (DEAE-Sepharose)	PCC	263K		WB	*3.3* [61]
IEC (DEAE-Sepharose)	FIX	263K	MF	WB	*3.0* [35]
Heparin-Sepharose	FIX	263K	MF	WB	*1.4* [35]
S-Sepharose	Thrombin (fibrin glue)	263K	MF	WB	*2.9* [35]

MF: microsomal fraction; WB: Western-blot.

Table 3
Extent of TSE agent removal during nanofiltration of plasma-derived coagulation factor concentrates. Adapted from Refs. [43,62]

Starting plasma fraction	Nanofilter	End-product	Agent	Spike	Assays	Reduction factor
DEAE-Toyopearl 650M eluate	Planova 35N	vWF	263K	MF	WB	*≥ 3.1* [61]
DEAE-Toyopearl 650M eluate	Planova 35N + 15N	FVIII	263K	MF	Bioassay	*≥ 3.3* [61]
SD + DEAE-Toyopearl 650M eluate	Planova 35N + 15N	FVIII	263K	MF	WB	*≥ 5.1* [61]
Monoclonal antibody chromatography eluate	Planova 75N + 35N + 35 + 15N	FVIII	263K	BH + SD	Bioassay	*4 + 0.3 + 1.3 + ≥ 0.5* [62]
Monoclonal antibody chromatography eluate	Planova 75N + 35N + 35N + 15N	FVIII	263K	PrPsc + SD	Bioassay	*3.1 + 0 + 0.8 + ≥ 2* [62]
Ion-exchange chromatography + heparin-Sepharose eluate	Planova 15N	FIX	263K	MF	Bioassay	*4.8* [61]
Ion-exchange chromatography + heparin-Sepharose eluate	Planova 15N	FIX	BSE 6PB1	BH + sonication	Bioassay	*5.3* [42]

MF: microsomal fraction; BH: brain homogeneate; WB: Western-blot; SD: solvent-detergent.

tate [39]. Using blood from scrapie-infected hamsters, and in spiking studies where scrapie 263K PrP^{sc} was added to human plasma, 20% and 10% of the infectivity partitioned in the cryoprecipitate, respectively [35,54]. By contrast, when human plasma was spiked with scrapie 263K brain homogeneate (BH), 90% of PrP^{sc} was found in the cryoprecipitate. These contradictory results may highlight the influence of the nature of the experimental model used, in particular the physicochemical nature of the spike [34], or of the variations in the down-scaling of the cryoprecipitation procedure, or they actually illustrate the fact that cryoprecipitation does not ensure robust partitioning of TSE infectivity.

3.3.2.2. Ethanol precipitation. The capacity of precipitation steps to remove prions efficiently has first been shown by partitioning of endogenous infectivity using a rodent model. It is particularly well documented for the ethanol fractionation process isolating albumin and immunoglobulins. In the albumin fractionation procedure using either the Cohn-Oncley or the Kistler and Nitchman processes, major and consistent reduction factors (typically 3–5 logs) of TSE agent have been found by various groups, most specifically during the precipitation of fraction II + III, fraction III, and fraction IV, or their equivalents using slightly modified fractionation conditions [35,42] (Table 4). Similar experiments revealed 3–5 logs removal during the precipitation III or I+III used in the IgG process [35,42]. These data suggest that, in spite of variations in the conditions (such as ethanol concentration and pH) used, reproducible clearance of PrP^{TSE} takes place. Removal is achieved when the precipitate is separated. It has been speculated that prion removal in precipitates is possibly due to aggregation and is dependent upon pH and presence of alcohol [35,55]. Other precipitation steps, using caprylic acid during immunoglobulins production [56] or polyethylene glycol also contribute to prion removal [42].

3.3.2.3. Depth filtration. Depth filters are made of a combination of a matrix (generally based on cellulose), filter aids (diatomeous earth, resins, or other adsorbents), and a drainage system. They are used to clarify crude protein solutions and

Table 4
Extent of TSE agent removal during the ethanol plasma fractionation process in the manufacture of albumin and immunoglobulin G. Adapted from Refs. [41,43,62]

Step	Fraction evaluated	Agent	Spike	Assays	Reduction factor (log_{10})
Precipitation of fraction I	Supernatant	263K	BH	WB	1.1 [41,43]
Precipitation of I + II + III	–	263K	MF	WB	1.3 [34]
Precipitation of I + II + III	–	263K	MF	WB	≥ 2.8[a] [42]
Precipitation of fraction II + III	–	263K	BH	WB	≥ 4.7 [41,43]
–	–	263K	BH	Bioassay	6.0 [41]
–	–	Sc237	BH/MF/CLD/PrPSc	CDI	3.6/3.1/3.1/4.0 [34]
–	–	263K	BH	WB	≥ 4.2/ ≥ 4.1 [41,43]
–	–	263K	BH	Bioassay	3.7/4.6 [41]
Precipitation of fraction I + III[b]	–	263K	MF	WB	≥ 3.5[a] [42]
Precipitation of fraction IV[c]	–	263K	MF	WB	≥ 3.0 [35]
–	–	Sc237	BH/MF/CLD/PrPSc	CDI	3.2/3.4/3.2/2.2 [34]
–	–	263K	MF	WB	≥ 3.7 [35]
–	–	263K	MF	WB	≥ 4.3[a] [42]
–	–	263K	BH	WB	≥ 4.3 [41,43]
–	–	263K	BH	Bioassay	5.3 [41]

BH: brain homogeneate; MF: microsomal fraction; CLD: caveolae-like domain; WB: Western-blot; CDI: conformation-dependent immunoassay.
[a] Evaluated together with filter aids to remove precipitates.
[b] Precipitate discarded during the manufacture of IgG.
[c] Precipitate discarded during the manufacture of albumin.

Table 5
Extent of TSE agent removal during depth filtration of albumin and immunoglobulin G fractions. Adapted from Refs. [41,43,62]

Fraction	Filter	Agent	Spike	Assays	Reduction factor (log_{10})
Supernatant I	Seitz Supra P80	Sc237	BH/MF	CDI	–0.1/0.1 [34]
Supernatant III	Millipore AP20	BSE 301V	MF	Bioassay	2.4 [37]
–	Seitz KS80P	BSE 301V	MF	Bioassay	≥3.1 [37]
–	Cuno Zetaplus	263K	BH	WB	≥3.3 [55]
Supernatant IV	Seitz Supra P80	Sc237	CLD/PrPsc	CDI	≥0.9/≥2.4 [34]
–	Seitz AKS5 (carbon)	263K	MF	WB	2.7 [42]
Fraction V (albumin)	Cuno Delipid-I	263K	MF	WB	2.3 [35]
–	Seitz KS80P	263K	MF	WB	≥4.9 [35]
Fraction II (IgG)	Seitz K200P	263K	MF	WB	≥2.8 [35]
–	Ca_3PO_4 + filter aid + Cuno	263K	MF	Bioassay	2.5 [42]
–	Cuno	263K	BH	Bioassay	≥4.9 [57]

BH: brain homogeneate; MF: microsomal fraction; CLD: caveolae-like domains; CDI: conformation-dependent immunoassay; WB: Western-blot.

Table 6
Extent of TSE agent removal during nanofiltration of plasma-derived albumin, and IgG. Adapted from Refs. [43,62]

End-product	Nanofilter	Agent	Spike	Assays	Reduction factor
Albumin[a]	Planova 35N	CJD	BH	Bioassay	≥5.9 [53]
Albumin[a]	Planova 35N	ME7	BH	Bioassay	4.93 [36]
Albumin + detergent[a]	Planova 35N	ME7	BH	Bioassay	1.61 [36]
Albumin[a]	Planova 15N	ME7	BH	Bioassay	≥5.87 [36]
Albumin + detergent[a]	Planova 15N	ME7	BH	Bioassay	≥4.21 [36]
RhO (D) IgG	VIRESOLVE 180	263K	Detergent treated, sonicated and filtered BH	WB	≥2.5 [63]
IgG	DV50	263K	BH	Bioassay	4.4 [64]
IgG	Planova 75N + 35N	263K	MF	Bioassay	3.2 [61]

BH: brain homogeneate; MF: microsomal fraction; WB: Western-blot.
[a] Nanofiltration is not used during production of albumin preparations; a detergent was added for experimental purposes only.

remove precipitates. As such they are an important adjunct to the ethanol precipitation process. Principle of action encompasses both removal of particulates larger than the pore-size by size-exclusion, and of smaller elements by adsorption. During immunoglobulin manufacture, the supernatant of Fraction III (Supernatant III) and the re-dissolved Fraction II precipitate, and during production of albumin, the Supernatant IV and the re-dissolved Fraction V, generally undergo depth filtration steps. Experimental spiking experiments (Table 5) have shown that several types and grades of depth filters can remove prions [35,37,42]. Depending upon the type of depth filter or the physico-chemical parameters of the suspension, PrP^{TSE} removal may be due to an aggregation in the presence of alcohol [55] or to hydrophobic adsorption on the filter aids [42]. The impact of protein composition and content remains to be investigated and understood to demonstrate and guarantee the robustness of this non-specific removal.

3.3.2.4. Nanofiltration. Table 6 shows experimental data on the removal of TSE agents during nanofiltration of albumin and IgG. As for coagulation factors, high reduction factors have been found. Prion partitioning is presumed to result from a size-exclusion mechanism.

3.4. Cleaning and sanitization

Most equipment, including stainless steel reactors, chromatographic gels and columns, ultrafilters are re-used and, therefore, must undergo steam (SIP) or chemical (CIP) sanitizing procedures between production batches. These processes should be validated to ensure proper bacterial, pyrogenic, viral, and protein decontaminations. Experience gained with the sterilization of surgical instruments has shown that autoclaving at 134 °C for 18 min or 121 °C for 30 min reduced the transmission of prion infectivity by a factor > 5 log_{10} [57, 58] but autoclaving without immersion is less effective (4–4.5 log reduction) [58]. Standard chemical decontamination methods (NaOH 1 N, NaOCl 20,000 ppm) and hydrogen peroxide alone achieved a reduction of > 6.5 and 4.5 log_{10}, respectively [58]. By experiments involving a hamster scrapie strain 263K BH model, it was shown that 0.1 M NaOH for 15 min, in the absence of detergent, at 4 and 18 °C caused a reduction of 3.5 and 4.0 log_{10} of the prion protein, respectively. In the presence of sarkosyl, a 60-min incubation in NaOH further enhanced PrPRES reduction to > or = 4.5 log_{10}, with no residual infectivity. Therefore 0.1 N NaOH could also effectively inactivate prions, and its efficacy can be enhanced by the addition of sarkosyl [59]. A separate study shows that 0.1 M NaOH at 60 °C for 2 min and 0.25 M NaOH at 30 °C for 60 min inactivate 3.96 and 3.93 log_{10} of mouse-adapted scrapie strain ME7, respectively, and 0.5 M NaOH at 30 °C for 60 or 75 min inactivates ≥ 4.23 and 4.15 log_{10} [60].

4. Conclusion

There is so far no evidence of transmission of prions by plasma derivatives. The prevalence of the disease in the population is considered to be very low, although possibly not quite as low as initially considered [5,6]. Based on experimental models, it is believed that the infectivity in the plasma does

not exceed a few infectious doses per ml. By lack of knowledge of the nature of the agent associated to the infectivity in plasma, and in the absence of validated screening tests, alternative precautionary measures have been introduced to prevent the possibility of transmission of vCJD by plasma derivatives. Epidemiological surveillance of the population is in place in countries where BSE and vCJD cases have been identified. In some countries, blood donation deferral criteria include travel or residence of donors in BSE countries, and history of previous transfusion or tissue transplantation. Based on filtration experiments of blood collected from scrapie-infected hamsters, leucoreduction decreases by about 50% the prion infectivity in plasma [22,27]. Extent of removal of TSE agents during the plasma fractionation process appears to be substantial. Data from various laboratories and using different experimental models show several logs removal of TSE infectivity during the fractionation process. The most effective, but non-specific, removal steps are ethanol precipitation, depth filtration, and ion-exchange chromatography. Nanofiltration was also demonstrated to remove several logs of TSE infectivity, possibly based on a specific prion removal mechanism by size-exclusion. Uncertainty on the validity of these experimental studies remains, and additional studies are needed, since the biochemical features of the infective agent in blood and plasma is not known, nor the extent to which it may be present in blood donations. Research should continue, aiming at identifying the features of TSE agents in human plasma and at ensuring the robustness of prion removal steps and sanitizing procedures during plasma product manufacture.

Acknowledgments

Thanks are expressed to Dr. Benoît Flan (Biological Safety Unit, LFB, France) for helpful expert discussions during the preparation of the manuscript.

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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5月7日	新医薬品等の区分 該当なし	機構処理欄
一般的名称	ﾃｸﾈﾁｳﾑ大凝集人血清ｱﾙﾌﾞﾐﾝ（^{99m}Tc）		研究報告の公表状況	ProMED-mail／20070423.1325(2007年4月23日) 1:Herald Sun online、2: Mailman School of Public Health, Columbia University, press release	公表国 オーストラリア	
販売名（企業名）	テクネMAA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	使用上の注意記載状況・その他参考事項等
オーストラリア（ヴィクトリア）において、一人のドナーから臓器提供を受けた3人（腎臓を移植された63歳女性、肝臓を移植された64歳の男性、腎臓を移植された3例目）の死因について、コロンビア大学とメルボルンの科学者らによって新たなウイルス（リンパ球性脈絡髄炎ウイルスに関連するアレナウイルス科）が発見された。しかしこのウイルスが単に付着しているだけか、組織拒絶と死の原因となるかどうかは未解決である。	特になし

報告企業の意見	今後の対応
臓器移植後死亡した3例の死因についての研究報告であり、これまで知られていない新ウイルス（リンパ球性脈絡髄炎ウイルスに関連するアレナウイルス科）が発見され、同科には出血熱性のラッサウイルスやマチュポウイルス等あることより、重大な感染症である可能性が考えられる。 本報告は、新たに判明した感染症に関するものであり、かつ、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等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。しかしこのウイルスが単に臓器に付着しているだけか、組織拒絶と死の原因となるかどうかは未解決であると報告されているため、今後も情報収集が必要であると考える。

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Archive Number 20070423.1325
Published Date 23-APR-2007
Subject PRO/AH/EDR> Arenavirus, organ transplants - Australia (VIC)

ARENAVIRUS, ORGAN TRANSPLANTS - AUSTRALIA (VICTORIA)
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A ProMED-mail post
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International Society for Infectious Diseases
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[1]
Date: Sun 22 Apr 2007
Source: Herald Sun online [edited]
<http://www.news.com.au/sundayheraldsun/story/0,,21598166-2862,00.html

Australia: Novel virus responsible for deaths of organ donation recipi
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A virus unknown to medical science was behind the deaths of 3 Victorians who received organs from the one donor. The unnamed bug has been linked to Ebola virus, [a virus] responsible for the deaths of thousands in central Africa since the 1970s. [This is an incorrect statement. The organ transplant-associated virus is not related to Ebola virus; see part [2] below. - Mod.CP]. After baffling local scientists, experts from New York's Columbia University were called in to help solve the mystery of the multiple transplant deaths being investigated by the coroner.

Initial investigations and tests had been unable to determine any common link between the donor and the 3 recipients. The presence of the virus in the recipients is thought to be a world first. One of the New York team said: "The discovery of this virus is of national and international significance."

The Sunday Herald Sun revealed the deaths in February 2007. A 63-year-old woman died after receiving a kidney transplant at Austin Hospital. A 64-year-old man died after receiving a liver transplant there. The 3rd victim received a kidney at Royal Melbourne Hospital.

The male donor whose organs carried the suspected killer bug had died in Dandenong Hospital of a brain hemorrhage in December 2006 after returning from overseas; it is believed most of his trip was spent in Europe.

The virus is part of the rodent-borne arenavirus family and can cause "old-world" diseases such as Yellow Fever, Ebola and Lymphocytic choriomeningitis. [This statement is incorrect: yellow fever is caused by a flavivirus and Ebola hemorrhagic fever is caused by a filovirus; only lymphocytic choriomeningitis (LCM) is caused by an old-world arenavirus. - Mod.CP]. Victoria's acting Chief Health Officer, Dr John Carnie, confirmed the virus [LCM virus?] had been detected in multiple samples from all 3 transplant patients. But there was no evidence the virus represented a public health risk, he said.

Health authorities are examining whether future donated organs can be screened for [LCM?] virus. A spokesman for the Victoria Coroner's office said families of the victims were told yesterday [21 Apr 2007]. There would be a formal inquest.

Experts from Columbia's Greene Infectious Diseases Laboratory helped

solve the mystery. Initial investigations and tests were unable to determine any common link between the donor and the 3 recipients. Dr Carnie said the risk to the public was minimal because "these viruses [?] affect immunocompromised people, and it is rarely fatal in those with normal immune systems. We have not had any indication of any unexplained illnesses among families of the donor or recipients," he said. "This would be the case if it was transmissible person to person. Our supposition is it was transmitted by organ transplantation."

Cutting edge techniques were used for the 1st time by the Greene lab -- in collaboration with Victorian Infectious Diseases Reference Laboratory -- to gene sequence the virus. "Our gene technology enables unbiased sequencing of all agents present," Columbia's Prof. Ian Lipkin said. "We found a handful (of combinations) that were related to Lassa virus or LCM virus [both old world arenaviruses - Mod.CP]. Using these clues we can confidently say this is a new virus, present in the original organs and so different than anything seen before."

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Communicated by:
ProMED-mail Rapporteur Brent Barrett

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[2]
Date: Sat 21 Apr 2007
Source: Mailman School of Public Health, Columbia University, press release [edited]
<http://www.prnewswire.com/cgi-bin/stories.pl?ACCT=104&STORY=/www/stor

Scientists Discover New Virus Responsible for Deaths of 3 Transplant Recipients From Single Donor in Victoria, Australia
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Knowledge of genetic sequence of virus will enable improvements in screening to enhance transplantation safety. Scientists in the Greene Infectious Disease Laboratory of Columbia University Mailman School of Public Health and colleagues in the Victoria Infectious Diseases Reference Laboratory in Melbourne, Australia and 454 Life Sciences have discovered a new virus that was responsible for the deaths of 3 transplant recipients who received organs from a single donor in Victoria, Australia.

The previously unknown virus, which is related to lymphocytic choriomeningitis virus (LCMV), was found using rapid sequencing technology established by 454 Life Sciences and bioinformatics algorithms developed in the Greene Laboratory with support from the National Institute of Allergy and Infectious Diseases. Known strains of LCMV have been implicated in a small number of cases of disease transmission by organ transplantation [see references below], however, the newly discovered virus is sufficiently different that it could not be detected using existing screening methods.

Over 30 000 organ transplants are performed in the U.S. each year. Knowledge of the genetic sequence of this virus will enable improvements in screening that will enhance the safety of transplantation.

Ian Lipkin, MD, director of the Greene Laboratory and Principal Investigator of the Northeast Biodefense Center, emphasized the importance of academic, public health, and industrial partnership in this work. "This was a team effort. Drs. Mike Catton and Julian Druce at the Victorian Infectious Disease Reference Laboratory reached out to us after a comprehensive state-of-the-art investigation failed to turn up leads," stated Dr. Lipkin. "We succeeded in identifying the virus responsible for the deaths by building on their work and utilizing new tools for pathogen surveillance and discovery developed in the Greene Laboratory and 454 Life Sciences."

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Communicated by: ProMED-mail <promed@promedmail.org>

[Lymphocytic choriomeningitis virtu (LCMV) is the type species of the genus _Arenavirus_ of the _Areanviridae_ family of bipartitie genome

RNA viruses. The reservoir hosts of almost all arenaviruses are rodents. LCMV is found in wild and laboratory mice, and other related "old world" arenaviruses are found in African species of rodents. Human LCMV infection may occur in rural and urban areas with high densities of rodents. Laboratory-acquired infections occur sporadically, and, previously, there have been a small number of cases of LCMV transmission by organ transplantation as mentioned by Professor Lipkin above. The virus detected by Professor Lipkin's group appears to be an LCMV-like agent but distinct from previously isolated strains of LCMV. It is unresolved, however, whether these organ-transplanted viruses are merely passengers or are responsible also for tissue-rejection illness and death. - Mod.CP]

[see also:
2005

LCMV, transplant recipients, fatal - USA (02) 20050526.1459
LCMV, transplant recipients, fatal - USA 20050524.1426
1995

LCMV & birth defects - USA 19951119.1095]
.....mpp/cp/msp/lm

別紙様式第2-1

No. 10

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. 4. 19	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		AbuBakar S, Sam IC, Wong PF, MatRahim N, Hooi PS, Roslan N. Emerg Infect Dis. 2007 Jan;13(1):147-9.	マレーシア

研究報告の概要

○マレーシアにおけるチクングニヤウイルス感染の再興
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には関与しないが、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。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
マレーシアで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、ウイルスのゲノム配列は他のインド洋諸国における流行株とは異なっていたとの報告である。	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。

Reemergence of Endemic Chikungunya, Malaysia

Sazaly AbuBakar,* I-Ching Sam,* Pooi-Fong Wong,* NorAziyah MatRahim,* Poh-Sim Hooi,* and Nuruliza Roslan*

Chikungunya virus infection recently reemerged in Malaysia after 7 years of nondetection. Genomic sequences of recovered isolates were highly similar to those of Malaysian isolates from the 1998 outbreak. The reemergence of the infection is not part of the epidemics in other Indian Ocean countries but raises the possibility that chikungunya virus is endemic in Malaysia.

Chikungunya, a mosquitoborne disease first described in Tanzania (formerly Tanganyika) in eastern Africa in 1952, is caused by chikungunya virus (CHIKV), an alphavirus belonging to the *Togaviridae* family. The disease occurs in Africa and various parts of Asia and is endemic in several southeast Asian countries, including Thailand, Indonesia, and the Philippines. Only 1 known outbreak has occurred in Malaysia, in 1998–1999 when ≥51 persons in Port Klang were infected (*1*).

From March through April 2006, an outbreak of CHIKV infection was reported in Bagan Panchor (4°31′N, 100°37′E), an isolated coastal town 50 km west of Ipoh, the state capital of Perak, in northwest Malaysia. At least 200 villagers were infected, with no deaths reported. This was the second known outbreak in Malaysia, 7 years after the previous one. This reemergence coincided with reports of ongoing epidemics of CHIKV infection in India and almost all the island nations of the Indian Ocean, with >200,000 cases in the French island of Reunion alone since February 2005 (*2*).

Why and how the recent infection reappeared in Malaysia remains unknown. The apparent absence of CHIKV for 7 years may be due to failure to detect low-level, continued transmission in humans, particularly because the symptoms may be mistaken for dengue fever. Alternatively, this outbreak could have originated from a viremic traveler from an endemic country (such as neighboring Thailand or Indonesia), but proximity of Malaysia to the Indian Ocean raises the possibility of an extension of the epidemic, with Malaysia being the furthest point yet of the expanding epidemic frontline.

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The Study

We received serum samples from 11 patients who had symptoms typical of CHIKV infection (Table). Samples were injected into Vero and C6/36 mosquito cells. Indirect immunofluorescence assays for immunoglobulin M (IgM) and IgG were performed using the patients' sera and CHIKV-infected cells fixed onto glass slides, as previously described (*1*). A CHIKV isolate (SM287) reported previously (*3*) was used to prepare the slides as a positive control for subsequent studies. Serum samples from patients who did not have symptoms of chikungunya, including patients with dengue fever, were used as negative controls. Nucleic acid amplification was performed using RNA extracted directly from the patients' sera or from cell cultures (Table). At least 3 different primer pairs specific for envelope glycoprotein E1 (E1), glycoprotein E2 (E2), and nonstructural protein 1 (nsP1) genes of CHIKV were used (*4,5*). Confirmation of the amplified DNA fragments was done by DNA sequencing. Phylogenetic relationships were examined using the E1, E2, and nsP1 gene sequences of the isolates and all other available CHIKV sequences obtained from GenBank or the previous studies (online Appendix Table, available from www.cdc.gov/ncidod/EID/13/1/147-appT.htm). Sequences were aligned and phylogenetic trees were drawn as previously described (*6*).

CHIKV infection was confirmed in 8 of 11 patients. CHIKV sequences were amplified directly from serum samples from 5 patients in the acute phase of disease. Of these, 4 CHIKV isolates were eventually cultured. IgM and IgG were detected in serum samples from 3 other patients in the convalescent phase (data not shown). In 1 patient, CHIKV sequences were amplified from serum samples obtained as late as 9 days after onset of symptoms (data not shown). The PCR amplification method, thus, could be useful for early detection of CHIKV infection in suspected outbreak situations.

The genomic sequence of the E1, E2, and nsP1 genes in the CHIKV isolates shared high similarity (>90%) to all the known CHIKV except West African CHIKV (≈86% similarity). The sequences were only ≈70% related to o'nyong-nyong virus, the most closely related alphavirus, which is present only in certain parts of Africa. Previous phylogenetic studies showed that CHIKV strains were clustered into 3 distinct groups based on origin from West Africa, Central/East Africa, or Asia (*7–13*). Phylogenetic trees drawn using E1 (Figure), E2, and nsP1 (data not shown) gene sequences clustered the recent Malaysian isolates into a group with other known CHIKV Asian isolates. The cluster, however, was distinctly separated (100% bootstrap support) from the African isolates and all the known isolates of the ongoing CHIKV epidemics of the Indian Ocean islands (*7–9,11,13*). This makes it unlikely that the outbreak in Malaysia is part of the ongoing epidemics,

Table. Identification of virus by PCR amplification and serologic analysis*

Patient		Chikungunya						Dengue fever	
		PCR†			Serology				Serology
Age (y)	Sex	E1	E2	nsP1	IgM	IgG	Culture	PCR‡	IgM
6	M	+	+	+	–	–	+§	–	–
34	M	+	+	+	–	–	+¶	–	–
40	M	+	+	+	–	–	+#	–	–
26	F	+	+	+	–	–	+**	ND	–
62	M	+	+	+	–	–	–††	ND	–
			(day 5 after onset)						
		–	–	–	+	+	ND	ND	ND
			(day 15 after onset)						

*IgM, immunoglobulin M; IgG, immunoglobulin G; +, positive; –, negative; ND, not determined.
†PCR amplifications were performed for detection of envelope glycoprotein E1 (E1), glycoprotein E2 (E2), and nonstructural protein 1 (nsP1) genes of chikungunya virus.
‡Multiplex PCR amplifications were performed for detection of dengue virus type 1–4.
§Isolate MY/0306/BP37348.
¶Isolate MY/0306/BP37350.
#Isolate MY/0306/BP37352.
**Isolate MY/0406/BP37437.
††Isolate MY/0306/BP34198.

despite its proximity to the region and timing of the outbreak. The phylogenetic tree, on the other hand, suggests that the isolates from the current Malaysia outbreak share a common ancestral lineage to the 2 Malaysian isolates recovered in 1998 (*4*; GenBank accession nos. AF394210 and AF394211) but have a slight genetic distance from all other Asian isolates.

Conclusions

On the basis of all available sequences of isolates from the neighboring countries where CHIKV is endemic, Thailand and Indonesia, the outbreak in Malaysia likely did not originate from either of these countries, which means the outbreak could have originated from an endemic CHIKV cycle not previously identified in Malaysia. A serologic survey of human serum samples collected during 1965–1969 in west Malaysia showed neutralizing antibodies to CHIKV among adults, especially those inhabiting the rural northern and eastern states bordering Thailand (*14*). The same authors also reported in an earlier study evidence of CHIKV-neutralizing antibodies in wild monkeys, a pig, and a chicken and suggested that a CHIKV sylvatic transmission cycle involving primates and possibly nonprimates exists in Malaysia. A sylvatic transmission cycle of the virus has been described in Africa and may play a role in the episodic emergence and reemergence of CHIKV infection (*15*). Before 1998, CHIKV had not been isolated from humans or animals in Malaysia, and no clinical disease caused by CHIKV had been reported. However, in the absence of active surveillance since the 1965 study, whether the apparent absence of CHIKV over the years and between the 2 recent outbreaks in Malaysia is due to an unidentified sylvatic transmission cycle or silent transmission among humans cannot be determined. Further investigation is required to examine these possibilities. Understanding this disease in Southeast Asia is critical because CHIKV shares the same mosquito vectors as dengue virus, which is endemic to the region.

Phylogenetic analysis showed that CHIKV from the recent 2006 outbreak in Malaysia is highly similar to isolates from the 1998 outbreak. At the 3 genes examined,

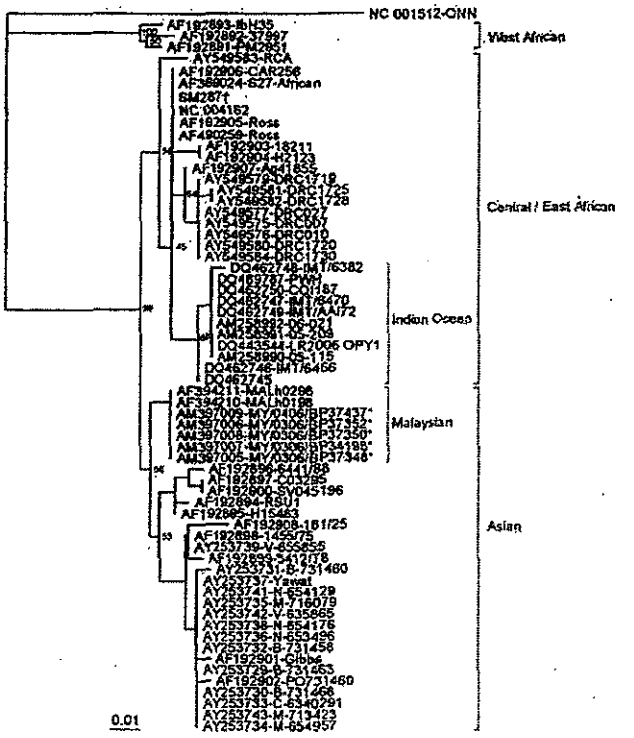

Figure. Phylogenetic relationships of chikungunya virus isolates from the 2006 Malaysia outbreak. The neighbor-joining tree was constructed using nucleic acid sequences of the envelope glycoprotein E1 gene, with o'nyong nyong virus (GenBank accession no. NC_001512) as the outgroup virus. * indicates isolates from the Malaysia 2006 outbreak; † indicates Australia SM287. Bootstrap values are shown as percentages derived from 1,000 samplings. The scale reflects the number of nucleotide substitutions per site along the branches.

the isolates differ from the ongoing Indian Ocean epidemic isolates and known isolates from Thailand and Indonesia. These findings support the possibility that the outbreak originated from an endemic infection in Malaysia.

Acknowledgments

We thank the staff of the Ministry of Health Malaysia and of the University of Malaya Medical Center, University of Malaya, Malaysia. David Smith from the Western Australian Center for Pathology and Medical Research, Perth, Australia, provided the CHIKV isolate (SM287).

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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. 4. 18	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況	Reuters AlertNet. 2007 Apr 13.	公表国	
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)			米国、ヨーロッパ	

研究報告の概要

○シャーガス病が輸血用血液を通じて米国やヨーロッパに拡大—WHO
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。バイエル社は250万錠のNifurtimox(販売名:Lampit)を寄贈した。これは、若者の急性症例を含め今後5年間に3万人の患者を治療できる量である。
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。シャーガス病は感染者に臓器の腫脹を引き起こし、最終的には死亡に至る病気で、正確な死亡率は不明である。大多数の感染者は、寄生虫を媒介する大型のナンキンムシに似た吸血昆虫に噛まれた後、感染していることを知らないまま数十年の潜伏期間を過ごすことになる。
「この病気はラテンアメリカの多くの人にとっては今でも脅威である。感染した供血者の適切なスクリーニングが行われていないため、血液銀行を通して脅威は他の国々に広がっている」とWHOの南北アメリカ担当者は話している。ジュネーブのWHO本部は、シャーガス病の撲滅に力を入れており、感染者数は1990年の1600～1800万人から減少してきた。
チリ、ウルグアイ、ブラジルの大部分、中米・アルゼンチン・ボリビア・パラグアイの広範囲の地域では、感染伝播は減少している。最も流行している地域は、ボリビアとアルゼンチンのチャコ地方、メキシコの一部、ペルー、コロンビアである。

報告企業の意見	今後の対応
シャーガス病が、ラテンアメリカから移住した人の供血を通して米国やヨーロッパに拡大しているとの報告である。	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。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

ıagas spread to U.S., Europe via blood banks -WHO

Apr 2007 13:47:09 GMT

urce: Reuters

NEVA, April 13 (Reuters) - Chagas, a parasitic disease which can kill victims decades after infection, has spread from Latin America to the ited States and Europe due to inadequate blood screening, the World Health Organisation said on Friday.

: United Nations agency said it was expanding its programme to eliminate Chagas, which has become a "global problem", with the help of /er HealthCare <BAYG.DE>.

/er's donation of 2.5 million tablets of Lampit, known generically as nifurtimox, will help treat an estimated 30,000 patients over the next five rs, covering new acute cases among youngsters, it said.

ıgas, which currently affects an estimated nine million people, mainly children in rural areas of Latin America, has emerged in the United tes, Spain and several other European countries after large-scale migrations, the WHO said.

exact death toll exists for the "silent killer" which causes the slow swelling of victims' internal organs, resulting in their eventual death, ording to the WHO.

st victims may not know they have contracted Chagas as the infection may remain dormant for decades after they have been bitten by a od-sucking insect similar to a large bed bug which transmits the parasite.

ıis disease still poses a threat to so many people in Latin America and now that threat has spread to other countries via blood banks lacking quate screening of infected donors," said Mirta Roses Periago, WHO director for the Americas region.

: Geneva-based WHO has been working to wipe out the disease and the number of those infected has fallen from 16-18 million people in 0.

nsmission of the disease has been interrupted in Chile, Uruguay, a large part of Brazil, as well as vast areas of Central America, Argentina, ivia and Paraguay, the WHO said.

: most endemic regions remain the Chaco regions of Bolivia and Argentina, as well as parts of Mexico, Peru and Colombia, according to ain.

REUTERS

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2007 年 5 月 7 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン		研究報告の公表状況	Transfusion 2007; 47(5): 883-889	公表国 アメリカ	
販売名 (企業名)	ハプトグロビン注ーヨシトミ(ベネシス)					

研究報告の概要

＜背景＞
特に凝固因子でのパルボウイルス B19（B19）は一般的な汚染物質である。1999 年に SD 処理を施したプール血漿による B19 伝播を理由として、幾つかの分画メーカーは製造プールの B19 負荷を制限するためにミニプール NAT を開始した。本研究では、B19 NAT スクリーニングが実施された前と後で製造された市販の第Ⅷ因子（AHF）製剤中の B19 DNA 汚染の程度を確認した。
＜研究デザイン及び方法＞
1993-1998 年及び 2001-2004 年の間に製造された 6 つの AHF 製剤を代表する全部で 284 ロットを、in-house の NAT 法により B19 DNA を測定した。抗 B19 抗体（IgG）も併せて測定した。
＜結果＞
1993-1998 年に製造されたほとんどのロットから B19 DNA が検出された。陽性率は 56～100％で、製造業者により異なっていた。検出された B19 DNA の最高濃度は 10^6 IU/mL であった。検査された 40％のロットは 10^3 IU/mL であった。対照的に、2001-2004 年の間に製造された原料血漿由来の AHF 製剤の陽性率及び濃度は低かった。しかしながら、回収血漿由来の製剤では変化が見られず、これはミニプール NAT が実施されていなかったためである。中間的な精製度の AHF 製剤のみが、抗 B19 抗体陽性であった。
＜結論＞
B19 NAT をスクリーニングしていない血漿から調製した AHF 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、製剤間でさまざまであった。血漿中の B19 NAT スクリーニングは、最終製剤中の B19 DNA レベルを下げて大半の例で検出限界以下とさせ、このことで B19 伝播のリスクを減少させた可能性がある。

使用上の注意記載状況・その他参考事項等

1. 慎重投与
(4) 溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(5) 免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1) 略
1) 血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
5. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

報告企業の意見	今後の対応
原料血漿におけるミニプール NAT の実施は、最終製剤中の B19 DNA レベルを下げ、B19 伝播のリスクを減少させる可能性があるとの報告である。 本ウイルスは血漿分画製剤の製造工程での不活化・除去が困難であり、その伝播リスクを完全に否定できないため、1996 年 11 月より、使用上の注意に本ウイルスの伝播リスクについての記載を行い、注意喚起を図っている。また、原料への本ウイルス混入量低減のため、RHA(Receptor-mediated Hemagglutination)法を用いたドナースクリーニングによる高力価血漿の排除を行なっている。	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。

Parvovirus B19 DNA in Factor VIII concentrates: effects of manufacturing procedures and B19 screening by nucleic acid testing

Yansheng Geng, Chuan-ging Wu, Siba P. Bhattacharyya, De Tan, Zheng-Ping Guo, and Mei-ying W. Yu

BACKGROUND: Parvovirus B19 (B19) is a common contaminant, especially in coagulation factors. Because of B19 transmission by pooled plasma, solvent/detergent treated in 1999, some fractionators initiated minipool nucleic acid testing (NAT) to limit the B19 load in manufacturing pools. In this study, the extent of B19 DNA contamination in commercial Factor VIII concentrates, that is, antihemophilic factor (human) (AHF), manufactured before and after B19 NAT screening was implemented, was determined.

STUDY DESIGN AND METHODS: A total of 284 lots representing six AHF products made during 1993 to 1998 and 2001 to 2004 were assayed for B19 DNA by an in-house NAT procedure. Anti-B19 immunoglobulin G (IgG) was also measured.

RESULTS: Most lots made during 1993 to 1998 had detectable B19 DNA. The prevalence ranged from 56 to 100 percent and appeared to differ between manufacturers. The highest level of B19 DNA found was 10^6 genome equivalents (geq or international units [IU]) per mL. Forty percent of the lots tested contained 10^3 geq (IU) per mL. In comparison, both prevalence and levels in source plasma–derived AHF products made in 2001 to 2004 were lower. Both, however, remained unchanged in the recovered plasma-derived product because B19 NAT screening had not been implemented. Only an intermediate-purity AHF product was positive for the presence of anti-B19 IgG.

CONCLUSION: The prevalence and levels of B19 DNA in AHF prepared from B19 NAT unscreened plasma were high but varied among products with different manufacturing procedures. B19 NAT screening of plasma effectively lowered the B19 DNA level in the final products and in the majority of cases rendered it undetectable and hence potentially reduced the risk of B19 transmission.

Parvovirus B19 (B19) is a small nonenveloped DNA virus, known to resist viral inactivation procedures commonly used in manufacturing of plasma derivatives; it is widespread among populations.[1] The prevalence of B19 viremia in blood and plasma donors has been reported to range from 0.003 to 0.6 percent, depending on the time of an epidemic or the sensitivity of nucleic acid testing (NAT) methods.[2-4] Extremely high viremic levels in plasma, for example, 10^{13} genome equivalents (geq) of B19 DNA per mL, are often found at an early phase of the infection in acutely infected but asymptomatic donors.[5] As a consequence, B19 DNA has been detected at high frequency and high levels in plasma pools and their resulting plasma derivatives, especially the coagulation products.[6-8] Reports of transmissions attributed to Factor (F)VIII concentrates

ABBREVIATIONS: AHF = antihemophilic factor (human); B19 = parvovirus B19; VI/R = viral inactivation/removal.

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The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be construed to represent any Agency determination or policy.

YG, DT, and ZG were supported by the Research Participation Program at the Center for Biologics Evaluation and Research administered by the Oak Ridge Institute for Science and Education through an interagency agreement between the U.S. Department of Energy and the Food and Drug Administration.

Received for publication July 18, 2006; revision received October 10, 2006, and accepted November 17, 2006.

doi: 10.1111/j.1537-2995.2007.01205.x

TRANSFUSION 2007;47:883-889.

(antihemophilic factor (human) [AHF]), subjected to solvent/detergent (S/D) treatment, heat treatment, or both, are numerous.[9-15]

Because of the B19 transmission in 1999 associated with pooled plasma, S/D-treated in a postmarket surveillance study that correlated product infectivity with a high concentration of virus in the manufacturing pool, plasma screening of B19 DNA by NAT in a minipool format was implemented as an in-process control test so that the viral load in the plasma pool used to manufacture the product could be limited to less than 10^4 geq per mL of B19 DNA.[16-19] The FDA has since proposed a similar limit for manufacturing pools destined for all plasma derivatives to reduce the potential risk of transmission.[19-23] Beginning in late 1999, some fractionators, mostly those who use source plasma, initiated (albeit gradually) the use of less sensitive, or so-called high-titer, minipool NAT screening to lower the viral load in manufacturing pools.[4,20-24] Some final products obtained from minipool-screened plasma have been found to be devoid of B19 DNA contamination.[4] The sensitivity of these minipool NAT tests varied but, in general, they excluded donations with B19 DNA levels of 10^6 geq per mL. In 2001, the Plasma Protein Therapeutics Association issued voluntary standards calling for manufacturers to implement 1) minipool screening of incoming plasma no later than the end of 2001 and 2) manufacturing pool testing to achieve levels of B19 DNA not to exceed 10^5 IU per mL no later than July 1, 2002.[23] Since then, all source plasma and manufacturing pools prepared from it have undergone B19 DNA testing.

The aim of this study was to evaluate the effect of B19 NAT screening of plasma on the resulting high-risk final products by comparing the prevalence and levels of B19 DNA in each of six US-licensed FVIII products made in two periods, that is, during 1993 to 1998 (before B19 NAT screening was implemented) and 2001 to 2004 (when such screening was nearly universal). Because the purification and viral inactivation/removal (VI/R) procedures used in the manufacturing of these products underwent little or no change over this entire span, the effectiveness of the B19 NAT screening could be evaluated, as could that of individual manufacturing procedures employed before any B19 screening.

MATERIALS AND METHODS

AHF samples

Six commercial AHF products represented by 136 lots made by five manufacturers during 1993 to 1998 and 148 lots made during 2001 to 2004, which were submitted by manufacturers to the FDA for lot release, were available for testing. The freeze-dried AHF products were reconstituted according to manufacturers' instructions, mostly with half of the specified volume, except that some lots made in 2004 were reconstituted with a full volume of the diluent. Unused reconstituted samples were stored at –70°C until further use.

DNA extraction and quantitation of B19 DNA by NAT

The extraction and semiquantitative NAT procedures were essentially the same as those described previously[15] except that a larger aliquot of reconstituted AHF, that is, 1.0 mL, was used for DNA extraction. For sample extraction and B19 NAT, either the WHO International Standard (NIBSC 99/800, 10^6 IU of B19 DNA/mL when reconstituted) or the CBER standard for B19 DNA (10^6 IU/mL) was used as a control.[25] Both were diluted 10^3-fold before use. Briefly, DNA from each sample or standard was extracted by use of an isolation kit and procedures (NucliSens, Organon Teknika, Durham, NC), and the DNA was recovered with 100 µL of the elution buffer. Aliquots of 25 µL of the undiluted or $10^{0.5}$-fold serially diluted DNA extracts in duplicate were used to perform nested polymerase chain reaction with primers derived from the VP1/VP2 region. Levels (in geq/mL) of B19 DNA in samples were determined by limiting dilution analysis. The sensitivity of the NAT assay for the large-volume extraction is 4 geq per mL, and the conversion ratio from geq to IU is 1:1.[15] This B19 NAT procedure detects both Genotype 1 and Genotype 2 of B19 but not the Genotype 3 variant (see Discussion).

Detection of anti-B19

Anti-B19 immunoglobulin G (IgG) was detected by use of a B19 IgG enzyme immunoassay kit (Biotrin International Ltd, Dublin, Ireland) according to the manufacturer's instructions except that a large sample aliquot, that is, 100 µL, was used for testing each reconstituted AHF. Most of the AHF samples tested were B19 DNA–positive.

Statistical analysis

The chi-square test was used to compare the prevalence between products. In addition, for comparing viral levels expressed as the log geometrical mean ± standard error of log geometrical mean (SEM), statistical analysis was performed by use of the unpaired t test. Results having p values of less than 0.05 were considered significant.

RESULTS

Prevalence and levels of B19 DNA in AHF lots manufactured during 1993 to 1998: effects of manufacturing procedures

Most products were made mainly from source plasma, but Product C was made from recovered plasma. The various purification and VI/R procedures used in the manufactur-

TABLE 1. B19 DNA prevalence and levels in FVIII concentrates made during 1993 to 1998*

Product	VI/R and purification	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots†						
			10^6	$\geq 10^5$	$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	Log geomean ± SEM
A	S/D, affinity, dry/80°C/72 hr	9/13 (69)	0	0	2	2	2	3	2.3 ± 0.41
B	S/D, immunoaffinity (SP)	22/25 (88)‡	0	0	7	10	3	2	3.0 ± 0.20§
C	S/D, immunoaffinity (RP)	14/15 (93)‡	0	2	2	8	2	0	3.3 ± 0.24§
D	S/D, gel filtration chromatography	15/15 (100)‡	2	6	1	2	3	1	3.9 ± 0.42§
E	Wet/60°C/10 hr	16/25 (64)	2	2	2	1	5	4	2.9 ± 0.45§
F	Wet/60°C/10 hr, immunoaffinity	24/43 (56)	0	0	0	3	11	10	1.7 ± 0.14
Total		100/136 (74)	4	10	14	26	26	20	

* Product C from recovered plasma (RP) and all other products from source plasma (SP); information regarding viral inactivation/removal (VI/R) and product purification procedures are from products' package inserts.

† B19 DNA–positive lots were categorized into groups, according to the level of B19 DNA. For 1993 to 1998, no positive lot was found to have fewer than 10 geq per mL; hence this category was not used. Categorization was done according to the following example: the at least 10^2 group indicates the number of positive lots containing less than 10^3 but at least 10^2 geq per mL. Levels in the last column are expressed as log geomean ± standard error of log geomean (SEM) among positive lots.

‡ $p < 0.01$ when compared to Product F; $p < 0.05$ when compared to Product E.

§ $p < 0.01$ when Product B, C, or D compared to Product F; $p < 0.05$ when Product E compared to Product F.

ing process are indicated in Table 1. Detectable B19 DNA in these products ranged from 56 to 100 percent of the lots tested (Table 1). The prevalence in either Product F or Product E was significantly lower than that in Products B, C, and D. Product F was a high-purity AHF product subjected to both wet heating at 60°C for 10 hours and purification by immunoaffinity chromatography utilizing monoclonal antibody (MoAb) to von Willebrand Factor (VWF), whereas Product E was an intermediate-purity product subjected only to wet heating. Interestingly, both Product B and Product C, which were S/D-treated, high-purity AHF products subjected to purification by immunoaffinity chromatography utilizing MoAb to FVIII, had a significantly higher positive rate than the wet-heated, high-purity Product F. In addition, the prevalences in lots of Products B and C, which were derived from source plasma and recovered plasma, respectively, were 88 and 93 percent, and hence there appeared to be no difference in prevalence of B19 as a function of the type of starting plasma. Three of the products, viz., A, E, and F, underwent manufacturing that included a heating step. When these were compared with the other products (B, C, and D), the prevalence was found to be significantly lower ($p < 0.001$), suggesting that heating was partially effective in eliminating detectable B19.

The highest level of B19 DNA in AHF products was 10^6 geq per mL found in 2 lots each of Products D and E (Table 1). Among 100 B19 DNA–positive lots, 54 lots (54% of the positive lots or 40% of the lots assayed) contained 10^3 geq per mL. Product F, which had the lowest prevalence of positive lots, also had the lowest B19 DNA levels among all products. The S/D-treated, immunoaffinity-purified Product B or Product C had significantly higher levels of B19 DNA when compared to Product F. There was no apparent difference in levels between Products B and C, which were derived from different types of starting plasma. Comparing the level of B19 DNA in heated product (A, E, and F) with that in unheated products (B, C, and D), suggested that heating was somewhat effective in lowering B19 contamination. Products E and F, however, differed significantly in B19 content ($p < 0.01$), despite the fact that both were heated for 10 hours at 60°C in solution during manufacture. Thus individual manufacturing steps (e.g., heating and immunoaffinity purification) can apparently be additive in their effects on B19.

Prevalence and levels of B19 DNA in lots manufactured during 2001 to 2004: effects of B19 NAT screening

To investigate the effect of minipool NAT screening by B19 NAT, we assayed AHF products made during 2001 to 2004 and compared the results with those from corresponding product lots made during 1993 to 1998. This appeared to be a valid comparison, inasmuch as the VI/R and product purification procedures for AHF products were essentially unchanged over this time span. The prevalence of B19 DNA in lots of products made from source plasma (i.e., all except Product C) ranged from 13 to 27 percent (Table 2). Moreover, all positive lots (25/129) made from source plasma contained less than 10^3 geq per mL, with 21 lots containing less than 10^2 geq per mL. Among 4 positive lots detected in Products A, B, and D, which had less than 10^3 but at least 10^2 geq per mL of B19 DNA, 3 lots were made in 2002, whereas 1 lot was made in 2004. In contrast, the prevalence in Product C, which was made from recovered plasma and had not been screened for B19 DNA, was 90 percent and the level of B19 DNA ranged as high as 10^4 geq per mL, with 9 of the 17 positive lots (53%) containing 10^3 geq per mL. Thus B19 NAT screening of plasma effectively lowered the B19 DNA level in all five products derived from source plasma, and in 81 percent of these 129 lots B19 DNA was undetectable. The product made

TABLE 2. B19 DNA prevalence and levels in FVIII concentrates made during 2001 to 2004

Product	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots*					
		$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	<10	Log geomean ± SEM
A	8/30 (27)	0	0	2	1	5	1.8 ± 0.54
B	3/24 (13)	0	0	1	2	0	1.6 ± 0.43
C†	17/19 (90)‡	2	7	5	2	1	2.9 ± 0.25§
D¶	4/16 (25)	0	0	1	1	2	1.4 ± 0.55
E	6/28 (21)	0	0	0	3	3	0.86 ± 0.14
F	4/31 (13)	0	0	0	0	4	0.54 ± 0.04
Total (C excluded)	25/129 (19)	0	0	4	7	14	

* See Table 1.
† See Table 1.
‡ $p<0.01$ when compared to Product B.
§ $p<0.01$ when compared to Product B.
¶ Product subjected to additional dry heating at 80°C for 72 hours during this period.

TABLE 3. Anti-B19 IgG in FVIII concentrates*

Product	Positive lots/lots tested	
	1993-1998	2001-2004
A	0/4	0/5
B	1/11	0/5
C	0/9	0/4
D	0/3	0/4
E	10/10	8/8
F	0/13	0/4

* For Products B and C, lots (made in 2001-2004) tested for anti-B19 were not exclusively B19 DNA–positive.

from unscreened recovered plasma exhibited not only a prevalence that was virtually unchanged over the two time periods but also levels of B19 DNA that were not significantly decreased.

Anti-B19 IgG in FVIII concentrates

A small sampling of lots (most of which were B19 DNA–positive) from each product was analyzed for the presence of anti-B19 IgG. B19 IgG was detected in all lots of Product E, an intermediate-purity AHF product whose manufacturing involved no chromatographic or affinity purification step (Table 3). In contrast, all other products except 1 lot from Product B were found negative.

DISCUSSION

During the mid and late 1980s, manufacturing procedures for FVIII concentrates changed greatly because of the inclusion of steps designed to improve product purity and/or to achieve VI/R. Since the early 1990s, however, the product purification and VI/R procedures for AHF products have remained largely unchanged. In this study, the prevalence and levels of B19 DNA in 136 lots representing six AHF products, that is, three high-purity products (Products B, C, and F) and three intermediate-purity products (Products A, D, and E), made in 1993 to 1998, were evaluated. Products B and C were made by an identical manufacturing procedure, whereas all other products were made by different methods.

B19 DNA has been reported to be prevalent in AHF products with levels as high as 10^7 geq per mL.[6,8] In our study, it was also found in the majority (74%) of the AHF lots made during 1993 to 1998, with levels of B19 DNA up to 10^6 geq per mL. Moreover, we found that the prevalence of B19 DNA was 100 percent in early AHF lots made in the 1970s (data not shown). The manufacture of these lots involved no VI/R steps, and B19 DNA levels sometimes reached 10^8 geq per mL. Our data are consistent with numerous reported B19 transmissions by FVIII concentrates subjected to either S/D or heat treatment or both.[9-15]

The wet-heated, high-purity Product F had the lowest prevalence and levels of B19 DNA among six products tested. Product E underwent a similar wet-heat treatment and also exhibited a low prevalence. It contained high levels of B19 DNA, however, apparently because its manufacturing lacks the immunoaffinity purification procedure used for Product F. An even more effective removal procedure for B19 might be developed by further exploiting this immunoaffinity-chromatography step (utilizing anti-VWF). Interestingly, for Product B or C, the immunoaffinity-chromatography step utilizing anti-FVIII has been validated and found to remove 4 logs of a model virus for B19, yet the prevalence and levels remained relatively high compared to those of Product F.

Products A, E, and F, all of which underwent manufacturing that included a heating step, had a significantly lower prevalence and B19 DNA levels than did unheated Products B, C, and D, suggesting that heating was partially effective in eliminating detectable B19. Product A was subjected to S/D treatment and affinity purification plus dry heating in the final container at 80°C for 72 hours. B19 is known to withstand dry heating at high temperatures, however, and transmissions have been documented in recipients of such heated AHF products.[11,12] The possible role of affinity purification in removing and hence lower-

ing B19 DNA in Product A cannot be ruled out because this method is used in combination with the dry heating. In contrast, our data for wet-heated Products E and F are consistent with the recent findings[26,27] that B19 can be susceptible to inactivation when heated in certain liquid media.

We also attempted to evaluate the possible effect of the type of plasma used on the prevalence and level of B19 in the resulting AHF product. This came about because Products B and C were derived exclusively from source plasma and recovered plasma, respectively, but made by the same manufacturing procedure. To obtain a product lot of comparable size, more units of recovered plasma are needed when compared to source plasma because of the difference in the volume of an individual unit. The results, however, suggest that there was no apparent difference because a similar prevalence and level of B19 DNA in the resulting product were obtained.

The prevalence of B19 DNA in blood and plasma donors can vary widely,[2-4,28] probably reflecting whether the collections were done at the time of an epidemic. The degree of B19 viremia in positive donations can also span a wide range,[4,28] with lower B19 DNA levels usually found in anti-B19 IgG–positive donors.[28] Likewise, before the implementation of B19 NAT screening, levels of B19 DNA in manufacturing pools were reported to range widely,[3,4,6-8] reaching as high as 10^9 geq per mL. These variations can, possibly, give rise to fluctuations in the levels of B19 DNA in final products—such as those observed in this study. Nonetheless, the consistent statistical differences in prevalence and level of B19 (Table 1) led us to conclude that individual manufacturing steps may have a significant effect in clearing, that is, inactivating and/or removing, B19.

The introduction of B19 NAT screening of source plasma after 1999 afforded us an opportunity to evaluate the effect of the viral load in the starting plasma on both the prevalence and the level of B19 DNA in the final product. In AHF lots made in 1993 to 1998 (when B19 NAT screening was not yet implemented), a total of 40 percent of the 136 lots tested, or 54 percent of the B19 DNA–positive lots, contained 10^3 geq per mL. In contrast, in AHF lots made in 2001 to 2004, B19 NAT screening of plasma effectively lowered B19 DNA levels in all five products derived from source plasma so that, of the 129 lots tested, *none* had a level of 10^3 geq per mL, and in 81 percent of the lots tested B19 DNA was undetectable. Obviously B19 NAT screening had not yet been implemented during this period for the recovered plasma destined for Product C, since the results were similar to those obtained from unscreened plasma in the earlier period. That is, 47 percent of the 19 lots tested, or 53 percent of the 17 positive lots, contained 10^3 geq per mL of B19 DNA.

Detection of B19 DNA in the product does not necessarily equate with infectivity. The actual infectious level of B19 in products is likely to depend on the level of anti-B19 IgG that is copresent in the product, in addition to the recipient's immune status. Anti-B19 IgG have been considered to be neutralizing antibodies and appear to confer lasting protection.[1] Although low viremic levels were found in donors who seroconverted to anti-B19 IgG,[28] seropositive sera have been shown by an in vitro infectivity assay system to contain neutralizing antibodies to B19.[29] Other studies[2,30] involving the infusion of B19 DNA–positive blood products with B19 DNA up to 2×10^6 geq per mL also demonstrated that when anti-B19 IgG was present either in the recipients or in the products, there was no B19 infection. In a recent publication,[31] it was reported that two seropositive immunocompetent recipients with anti-B19 IgG levels of 19 and 39 IU per mL were not infected with B19 after receiving pooled plasma, S/D-treated containing high-titer B19 DNA (1.6×10^8 IU/mL) and anti-B19 IgG (72 IU/mL), whereas the seronegative recipients were infected. These studies have strongly suggested the protective role of anti-B19 IgG.

In our study, none of the products tested, with the exception of the intermediate-purity Product E and one lot of Product B, had detectable anti-B19 IgG. In view of the fact that at least 50 percent of adults have circulating anti-B19 IgG,[32,33] B19 antibodies will invariably be present in any large plasma pool. Interestingly, most manufacturing procedures for AHF must be effective in separating FVIII from IgG. A product containing B19 DNA but devoid of anti-B19 IgG would potentially be infectious in seronegative recipients.

The minimal infectious dose (in terms of B19 DNA) in seronegative individuals is unknown. In a recent case report, we found that infection occurred when a seronegative, immunocompetent patient with mild hemophilia received an S/D-treated, high-purity AHF product, which contained 10^3 geq per mL of B19 DNA and was devoid of any detectable anti-B19 IgG; the total dose infused was 2×10^4 geq.[15] This is the lowest infectious dose (in terms of B19 DNA) that has been reported for a product containing no anti-B19 IgG. In a separate transmission case, a seronegative child was also infected by infusing a dry-heat-treated FVIII concentrate, which contained 4×10^3 geq per mL B19 DNA, but the total dose in terms of B19 DNA was 4×10^6 geq, and the product's anti-B19 IgG content, if any, was unknown.[14] For pooled plasma, S/D-treated, which was anti-B19 IgG–positive, a higher dose of B19 DNA was needed to produce infection. Only those seronegative volunteers infused with a 200-mL dose of product lots containing more than 10^7 geq per mL B19 DNA were infected, whereas those infused with an equal volume of lots containing less than 10^4 geq per mL did not seroconvert.[16,17,19] It remains to be determined whether AHF products containing low residual levels of B19 still may transmit the infection to susceptible individuals (presumably seronegative persons), especially to those high-risk individuals who are immune deficient.

B19 virus isolates have recently been classified into three genotypes with the majority of the isolates grouped as Genotype 1.[34] Like Genotype 1, Genotype 2 DNA was detected in FVIII concentrates as a contaminant but with lesser prevalence (2.5%); interestingly all Genotype 2-positive lots were also Genotype 1-positive.[35] The infectivity of a B19 Genotype 2 virus was recently found to be similar to that of Genotype 1 in an in vitro assay.[36] Genotype 3 has not been reported in FVIII concentrates. The lack of such a report, however, may be due to the fact that some B19 NAT procedures detect only Genotype 1 but not variant Genotype 2 or 3.[37] The NAT procedure used in this study detects both Genotype 1 and Genotype 2 but does not detect Genotype 3.

In conclusion, we have demonstrated that the prevalence and levels of B19 in FVIII concentrates made from plasma that was not screened for B19 DNA were high but varied among products with different manufacturing procedures. Minipool NAT screening for B19 DNA effectively lowers the prevalence and level of B19 in AHF. The majority of the lots of AHF now being manufactured have no detectable B19 DNA, and thus the risk of B19 transmission may be greatly reduced.

ACKNOWLEDGMENTS

We thank J.S. Finlayson, PhD, for critical review of the manuscript. We acknowledge AHF manufacturers for providing some samples and the lot-associated information when needed.

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識別番号・報告回数			報告日	第一報入手日 2007 年 5 月 22 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	人ハプトグロビン		研究報告の公表状況	Journal of virology 2007; 81(8): 4130-4136	公表国 スウェーデン	
販売名 (企業名)	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

我々は先に、臨床サンプルについての大規模な分子的なウイルススクリーニングのシステムを報告した。まだ発見されていないヒトの病原体を組織的に探索する努力の一環として、この技術を、ヒトの気道からの検体をもとにしたウイルスのスクリーニングに適用した。その結果、以前には知られていなかった KI ポリオーマウイルスと暫定的に名付けた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樹的に近似するが、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（アミノ酸の 30%未満が同一）。このウイルスは、PCR によって、鼻咽頭吸引物の 6/637 (1%) と便検体の 1/192 (0.5%) に見出されたが、尿及び血液の検体には見出されなかった。ポリオーマウイルスは発癌の可能性を有し、免疫が抑制されたヒトに重篤な疾患を起こす可能性があることから、様々な医療状況下で、このウイルスを探索し続けることが重要である。

報告企業の意見	今後の対応
ヒトの気道からの検体から、以前には知られていなかった KI ポリオーマウイルスと暫定的に名付けたポリオーマウイルスを同定したとの報告である。なお、このウイルスと近似のウイルスの発見報告を BENE2007-013 で報告する。 ポリオーマウイルスは、直径 40nm のエンベーロープを有しない DNA ウイルスである。万一原料血漿にポリオーマウイルスが混入したとしても、EMC 及び CPV 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

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-Ⅰ抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

JOURNAL OF VIROLOGY, Apr. 2007, p. 4130–4136 Vol. 81, No. 8
0022-538X/07/$08.00+0 doi:10.1128/JVI.00028-07

Identification of a Third Human Polyomavirus▽

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Received 5 January 2007/Accepted 30 January 2007

We have previously reported on a system for large-scale molecular virus screening of clinical samples. As part of an effort to systematically search for unrecognized human pathogens, the technology was applied for virus screening of human respiratory tract samples. This resulted in the identification of a previously unknown polyomavirus provisionally named KI polyomavirus. The virus is phylogenetically related to other primate polyomaviruses in the early region of the genome but has very little homology (<30% amino acid identity) to known polyomaviruses in the late region. The virus was found by PCR in 6 (1%) of 637 nasopharyngeal aspirates and in 1 (0.5%) of 192 fecal samples but was not detected in sets of urine and blood samples. Since polyomaviruses have oncogenic potential and may produce severe disease in immunosuppressed individuals, continued searching for the virus in different medical contexts is important. This finding further illustrates how unbiased screening of respiratory tract samples can be used for the discovery of diverse virus types.

Persistent virus infections are an integrated part of human life. Most humans are persistently infected with one or more herpesviruses, papillomaviruses, polyomaviruses, and anelloviruses and remain healthy. Nevertheless, many of these viruses may occasionally produce severe disease (21, 32, 34, 37). Identification of previously unrecognized viral species is technically difficult. Thus, many potentially medically important persisting human viruses most likely remain undetected.

Polyomaviruses are small DNA viruses capable of persistent infection and having oncogenic potential. They have been found in many mammals and birds worldwide. Two polyomaviruses are known to normally infect humans, JC virus (JCV) and BK virus (BKV), both discovered in 1971 (13, 30). They are genetically closely related to each other, and both viruses show 70 to 80% seroprevalence in adults (23). The routes of acquisition and sites of primary infection are largely unknown, but both viruses can establish a latent infection in the kidneys and, in the case of JCV, also in the central nervous system (31). Persistent replication in the kidneys is evidenced by the fact that JCV, and occasionally also BKV, can be detected in the urine of healthy adults (23). BKV has also been detected in the feces of children (35). JCV and BKV are highly oncogenic in experimental animals, but a role in the development of human tumors has not been established (25). Disease caused by human polyomaviruses has been observed in immunosuppressed individuals. JCV is the causative agent of progressive multifocal leukoencephalopathy, a demyelinating disease of the brain and a feared complication of AIDS (21). This disorder has recently received renewed attention after the occurrence of fatal cases among patients treated with natalizumab for multiple sclerosis (22, 24). BKV has been associated with post-transplantation nephropathy and hemorrhagic cystitis in hematopoietic stem cell transplant (HSCT) recipients (7, 17). In addition to JCV and BKV, there are reports on the presence of the primate polyomavirus simian virus 40 (SV40) in humans, possibly introduced by contaminated poliovirus vaccine produced in monkey cells (4), although other ways of transmission have also been suggested (10, 27). SV40 genomic sequences have been detected in human malignant mesothelioma tumors, but its role in human tumor development remains debated (25).

We have developed a system for large-scale molecular screening of human diagnostic samples for unknown viruses (2). With this technology, we have initiated a systematic search for previously unrecognized viruses infecting humans in order to identify agents that are potentially involved in human disease. We describe here the identification and molecular characterization of a hitherto unknown human polyomavirus, which is only distantly related to the other known primate polyomaviruses. In analogy with the nomenclature of the other human polyomaviruses, we propose the name KI polyomavirus, KIPyV, for the newly discovered virus.

MATERIALS AND METHODS

Molecular virus screening. As part of a systematic search for unknown viruses in clinical respiratory tract samples, a screening library was constructed from cell-free supernatants of 20 randomly selected nasopharyngeal aspirates made anonymous and submitted to the Karolinska University Laboratory, Stockholm, Sweden, for the diagnosis of respiratory tract infections. The samples were collected from March to June of 2004 and stored at −80°C until analyzed. This study was approved by the Karolinska Institutet local ethics committee. The procedure used for identification of virus nucleic acid sequences, molecular virus screening, has been described previously (2). In brief, samples were pooled and the pool was divided into two aliquots, which were filtered through 0.22- and 0.45-μm-pore-size disc filters (Millex GV/HV; Millipore), respectively. Both aliquots were ultracentrifuged at 41,000 rpm in an SW41 rotor (Beckman) for 90

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▽ Published ahead of print on 7 February 2007.

min. The resulting pellet was recovered, resuspended, and treated with DNase before DNA and RNA were extracted (1). Extracted DNA and RNA were amplified separately by "random PCR" (2, 12). The amplification products were separated on an agarose gel, and fragments between approximately 600 and 1,500 bp in length were cloned. A total of four libraries were generated, derived from DNA or RNA, and filtered through a 0.22- or 0.45-μm filter. Ninety-six clones from each library were sequenced bidirectionally, i.e., a total of 384 clones. A set of specially designed C++ and Perl programs were used for automated quality trimming, clustering, BLAST searches, sorting, and formatting of the sequence reads. The output was a sorted list of the best database hits for nucleotide and translated sequences.

Genomic analysis of the KIPyV genome. A 4,808-bp-long PCR product reaching around the circular DNA genome was generated by primers directed "outward" from the first cloned fragment (Pol-82R [TTGACTTCTTGGCCTTGTTAG] and Pol-315F [AGATGCTGACACAACTGTATG]) and by using a long-range enzyme mixture (Platinum *Taq* High Fidelity; Invitrogen). A second PCR product of 500 bp overlapping both ends of the long product and closing the circle was generated by primers PolconF (GGATTTTGTATGTGCTAGAAC) and PolconR (TTAACTAGAGGTACAACAAGC). Both PCR products were directly sequenced in order to obtain a consensus sequence for the complete genome. The same procedure was applied for determining the full-length sequences of three isolates. Putative open reading frames (ORFs) were identified, and sequences were aligned with Clone Manager Suite 6 (version 6.00) and Align Plus (version 4.10) (Scientific and Educational Software, Durham, NC). Prediction of putative binding sites for transcription factors was performed by comparison with consensus sequences and with the help of the AliBaba software (version 2.1) (16).

Phylogenetic analysis. All sequences were downloaded from GenBank, except those of murine pneumotropic virus, which were based on a corrected sequence (T. Ramqvist, unpublished data). Accession numbers are available upon request. The complete genomes and the amino acid sequences of the early and late proteins, respectively, were aligned and neighbor-joining trees generated with ClustalX version 1.83. The data were bootstrapped with 1,000 replicates, and trees were viewed with NJplot. For whole-genome analysis, the noncoding control regions were removed in accordance with established conventions and the first nucleotide in the T antigens was designated nucleotide 1.

PCR for detection of KIPyV. PCR experiments for detection of KIPyV were performed in a diagnostic laboratory setting, ensuring that the necessary precautions to avoid contamination were taken. Positive and negative controls were included in each experiment. DNA was extracted by commercially available kits as described under the respective sample type. Five microliters of extracted DNA was used as the template for the nested PCR. The 50-μl reaction mixtures used for the first and second PCRs consisted of 1× GeneAmp PCR buffer II (10 mM Tris-HCl [pH 8.3], 50 mM KCl; Applied Biosystems), 2.5 mM $MgCl_2$, 0.2 mM each deoxynucleoside triphosphate, 2.5 U of AmpliTaq Gold DNA polymerase (Applied Biosystems), and 20 pmol of each of the primers. The first-PCR primers were POLVP1-39F (AAG GCC AAG AAG TCA AGT TC) and POLVP1-363R (ACA CTC ACT AAC TTG ATT TGG). The second-PCR primers were POLVP1-118F (GTA CCA CTG TCA GAA GAA AC) and POLVP1-324R (TTC TGC CAG GCT GTA ACA TAC). The cycling conditions for the first and second PCRs were 10 min at 94°C, followed by 35 cycles of amplification (94°C for 1 min, 54°C for 1 min, and 72°C for 2 min). Products were visualized on an agarose gel. The product size after the second PCR was 207 bp. All PCR products were sequenced in order to confirm that they were specific for KIPyV.

Prevalence study populations. (i) Nasopharyngeal aspirates. Six hundred thirty-seven stored nasopharyngeal aspirates submitted to the Karolinska University Laboratory for diagnosis of respiratory virus infections from July 2004 to June 2005 were studied. Sampling month, patient's age and sex, and routine diagnostic (immunofluorescence and virus culture) findings were recorded before samples were made anonymous. The median age of the sampled patients was 7 years (range, 0 months to 90 years). Two hundred seventy-one samples came from children <2 years old. Total nucleic acids were extracted from 200-μl samples by the MagAttract Virus Mini M48 kit (QIAGEN), and nucleic acids were eluted in 100 μl. Eluted nucleic acids were initially analyzed in pools of 10 samples, and 5 μl of the pool was used as the template for the PCR. Single samples from PCR-positive pools were analyzed.

(ii) Feces. One hundred ninety-two fecal samples submitted to the Karolinska University Laboratory for diagnosis of virus infections from 1 July 2005 to 30 November 2005 were studied. Samples were mainly submitted for diagnosis of gastroenteritis. Basic sampling data were recorded before samples were made anonymous. The median age of the sampled patients was 1 year (range, 0 months to 17 years). One hundred nineteen samples came from children <2 years old. Nucleic acids were extracted from 400 μl of a frozen 20% feces suspension by MagAttract Virus Mini M48 kit and the Biorobot M48 instrument (QIAGEN) and eluted in 100 μl, and 5-μl samples were used for subsequent individual PCR assays.

(iii) Urine of HSCT recipients. One hundred fifty urine samples collected from HSCT recipients for the study of BKV and JCV were analyzed (14). Fifty of the samples were selected on the basis of previous analysis results; 20 were previously shown to be positive for BKV, 8 were positive for JCV, 2 were positive for both BKV and JCV, and 20 were negative for both viruses. JCV and BKV status was unknown for the remaining 100 samples. As described previously, samples were analyzed by PCR without preceding DNA extraction (6).

(iv) Serum of HSCT recipients. Thirty-three serum samples drawn from 17 HSCT recipients 2 to 6 weeks after transplantation were studied. Total nucleic acids were extracted from 200 μl of serum by QIAamp Virus Spin Kit (QIAGEN) and eluted in 50 μl.

(v) Whole blood. Whole EDTA blood from 192 healthy volunteer blood donors in Stockholm was analyzed. DNA was extracted from 200-μl samples with the MagAttract DNA Mini M48 kit and the Biorobot M48 instrument (QIAGEN) and eluted in 50 μl.

(vi) Leukocytes. Ninety-six frozen preparations of Ficoll-separated leukocytes were studied. Samples were originally sent to the laboratory for diagnosis of cytomegalovirus by PCR and virus culture and therefore mainly originated from immunosuppressed patients. DNA was extracted from 10^5 cells with the MagAttract DNA Mini M48 kit and the Biorobot M48 instrument (QIAGEN) and eluted in 100 μl.

Nucleotide sequence accession numbers. The sequences reported in this paper have been deposited in the GenBank database under accession no. EF127906 (KIPyV isolate 60), EF127907 (KIPyV isolate 350), and EF127908 (KIPyV isolate 380).

RESULTS

Molecular virus screening of 20 respiratory tract samples. A virus-enriched DNA-cDNA library was constructed from 20 randomly selected nasopharyngeal aspirate samples by a previously published protocol (2). After vector and low-quality sequences were automatically discarded, sequence reads from 374 (97%) clones remained for database searches. By automated nucleotide and translated BLAST searches (3), the sequences were categorized as likely (expected value, $<10^{-4}$) human (73%), bacterial (5%), phage (1%), unknown (2%), and virus (20%) sequences. Sixty-nine of the 74 clones with viral sequences matched human rhinovirus or enterovirus species. Reliable discrimination of rhinovirus from enterovirus sequences or type determination could not be performed on the basis of the unassembled sequence reads. Five clones closely matched respiratory syncytial virus. In addition to these virus-like clones, a single clone of 363 bp showed weak amino acid similarity (30% identity, expected value = 0.011) to VP1 of SV40 and was selected for further studies.

Genome analysis of KIPyV. The source nasopharyngeal aspirate sample containing the SV40-like sequence was identified by PCR analysis of aliquots saved before pooling. The positive sample was named Stockholm 60. A second PCR product reaching around the circular DNA genome was used as a template for determining the complete consensus viral genome sequence. The genome was confirmed to be circular and 5,040 nucleotides in length (accession number EF127906). Two additional isolates that were identified during the subsequent prevalence study (see below) were sequenced by the same approach. (Stockholm 350, accession number EF127907; Stockholm 380, accession number EF127908). The three genomes were highly similar. Both isolates Stockholm 350 and Stockholm 380 differed from the prototype isolate by 10 nucleotide substitutions, and they differed from each other by seven single bases. The variable positions showed some clus-

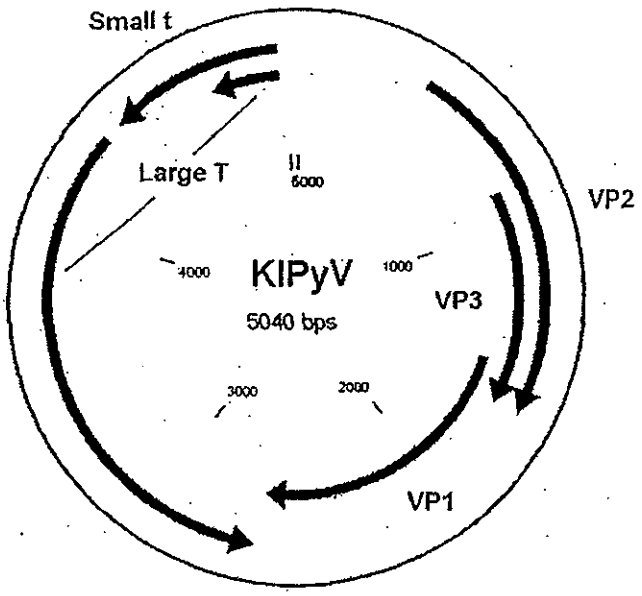

FIG. 1. Genome organization of KIPyV. Putative coding regions for VP1 to VP3, ST antigen, and LT antigen are marked by arrows.

tering in the regulatory region, but there were also a few isolate-specific amino acid substitutions in the putative proteins.

Overall genome organization. The genomic organization of KIPyV is typical for a member of the family *Polyomaviridae*, with an early region encoding regulatory proteins (small t [ST] and large T [LT] antigens) and a late region coding for structural proteins separated by a noncoding regulatory region (Fig. 1). The genome size is within the range of polyomaviruses. Properties of the deduced proteins and their similarities to those of JCV, BKV, and SV40 are shown in Table 1. While the nonstructural proteins have substantial amino acid sequence similarity to those of the other primate polyomaviruses, the structural proteins have a very low degree of similarity to those of other known polyomaviruses.

Regulatory region. The noncoding regulatory region of polyomaviruses contains the promoters for early and late gene transcription, origin of replication, and transcriptional enhancers. The core origin of replication of KIPyV contains three potential LT antigen binding sites, compared to the four found in most polyomaviruses (Fig. 2). Two of these have the classical sequence GAGGC, while the third has the sequence GGGGC. An A/T-rich domain, probably harboring a TATA box for the early mRNA, lies to the late side of these binding sites. To the early side of the binding sites, polyomaviruses normally contain an imperfect palindrome, followed by additional LT antigen binding sites. KIPyV has three additional potential LT antigen binding sites where expected, but the there is no palindrome pattern. In fact, no long palindromes or repeats were found in the regulatory region of KIPyV. Putative binding sites for transcription factors were predicted in silico. Transcription factors with more than one putative binding site were c-Ets-1, Oct-1, and NF-1 (Fig. 3). In addition, there were transcription factors with a single putative binding site (data not shown). No binding sites for Sp1 could be found.

Early region. In the early region of the genome, there are putative ORFs for the two regulatory proteins ST antigen and LT antigen (Fig. 1). On the basis of consensus sequences and alignments with LT antigens of other polyomaviruses, we propose a donor splice site for LT antigen at position 4716 and an acceptor site at position 4328. No middle T (MT) antigen seems to be expressed on the basis of the following. (i) Both polyomaviruses that express the MT antigen (murine polyomavirus [MPyV] and hamster polyomavirus) use all three reading frames for synthesis of the ST, LT, and MT antigens, while all other polyomaviruses, KIPyV included, use either one or, in some cases, two. (ii) In both MPyV and hamster polyomavirus, the MT antigen mRNA is produced through splicing, and no corresponding splice sites have been found in KIPyV. Assuming there is no expression of MT antigen, tiny T antigen can most likely not be expressed either (33).

The early proteins show similarities to other members of the polyomavirus family, primarily BKV, JCV, SV40, and simian agent 12 (SA12), and alignment with the LT antigens of other polyomaviruses shows that most regions characteristic of LT antigen are present also in KIPyV. The N-terminal 82 amino acids (aa) of the ST antigen are common to the LT antigen. This region encompasses the J domain carrying the conserved region 1 sequence and the HPDKGG box. In the C-terminal part that is unique to the ST antigen, there is a cysteine-rich domain typical of polyomaviruses. In the LT antigen, the HPDKGG box is followed by a putative Rb binding domain (LRCNE), a nuclear localization signal, a DNA binding domain, a Zn finger region including the zinc finger motif (C-312, C-315, H-327, H-331), and finally an ATPase-p53 binding domain containing the highly conserved GPXXXGKT sequence (aa 434 to 441). Unlike BKV, JCV, SV40, and SA12, the host range domain seems to be missing.

Late region. In the late region of the genome, there are putative ORFs for capsid proteins VP1, VP2, and VP3 (Fig. 1). As in all polyomaviruses, VP3 is encoded by the same ORF as VP2 by the use of an internal start codon. There is an overlap between the C terminus of VP2/3 and the N terminus of VP1, as is the case in other polyomaviruses. It can be noted that both VP2 and VP3 of KIPyV are large in comparison with those of other members of the polyomavirus family (400 and 257 aa, respectively).

For VP1, there is only one possible start codon, in contrast to the VP1 proteins of BKV, JCV, and SV40. The degree of homology with other VP1 proteins is remarkably low (Table 1). VP1 has only 30% identity with its closest counterparts (those of JCV and MPyV). In KIPyV VP1, the only region that shows

TABLE 1. Putative proteins encoded by KIPyV[a]

Protein	Putative coding region(s)	No. of amino acids	Calculated mass (kDa)	Amino acid identity (%) with:		
				BKV	JCV	SV40
VP1	1498–2634	378	41.6	29	30	29
VP2	441–1643	400	41.8	22	23	22
VP3	870–1643	257	28.2	22	24	22
ST antigen	4967–4392	191	23.2	37	36	40
LT antigen	4967–4716, 4328–2655	641	74.3	48	47	47

[a] Molecular masses were calculated by means of the ProtParam web tool (http://www.expasy.ch/tools/protparam.html).

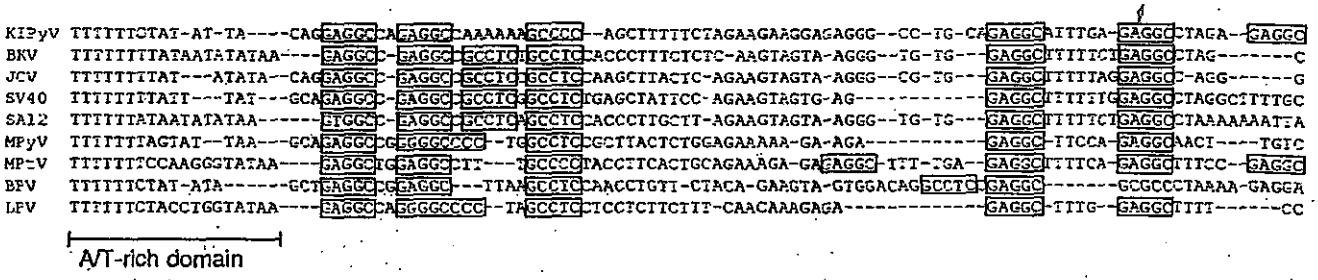

FIG. 2. Alignment of the origins of replication of nine polyomavirus species. Putative binding sites for LT antigen are boxed. MPtV, murine pneumotropic virus; BPV, bovine polyomavirus; LPV, lymphotropic papovavirus. This alignment is a modified form of that of Mayer and Dörries (26).

a relatively high degree of similarity to those of other polyomaviruses is the sequence that in MPyV VP1 has been shown to bind calcium, corresponding to approximately aa 237 to 248 in VP1 of KIPyV. Otherwise, VP1 of KIPyV has very limited homology to those of other polyomaviruses.

The VP2/VP3 gene showed even lower similarity to its counterparts in other polyomavirus species (Table 1). In fact, neither a nucleotide nor a translated BLAST search with this gene sequence generated any significant matches in the public databases. Thus, the identity of this ORF is only indicated by its position in the genome. VP2 and VP3 of all other polyomaviruses contain a conserved VP1 binding domain (located at approximately aa 281 to 295 in MPyVP2). No corresponding sequence is found in KIPyV.

Several polyomaviruses such as BKV, JCV, and SV40 express an Agno protein from the late mRNA. In KIPyV, the region between the start codons of VP2 and ST/LT, respectively, is large (513 bp) and this could possibly indicate the presence of an *agno* gene. However, there is no corresponding ORF present in this region.

Phylogenetic analysis. Phylogenetic trees were constructed on the basis of alignments of the first isolate, Stockholm 60, with known viruses of the *Polyomaviridae* family. Analysis of early protein genes consistently clustered KIPyV with JCV, BKV, SV40, and SA12 but as an outlier in this clade (Fig. 4). Analysis of the complete genome yielded highly similar results (data not shown). In contrast, analysis of the late protein genes consistently placed KIPyV outside the tree as the most distant group member (Fig. 4).

Prevalence of KIPyV in human samples. In order to investigate possible replication sites and suitable sample materials for prevalence studies, several sample sets were investigated for the presence of KIPyV DNA by a PCR targeting the gene for VP1 (Table 2). The identities of the PCR products were confirmed by sequencing. The results were also confirmed by a second PCR assay targeting the LT antigen gene. KIPyV was detected in nasopharyngeal aspirates and feces but not in urine, whole-blood, leukocyte, or serum samples (Table 2). Two isolates obtained from nasopharyngeal aspirates were fully sequenced. The ages of the six subjects positive for KIPyV in the nasopharynx ranged from 1 month to 26 years (median, 2 years), and the subject with positive feces was 3 months old. In five of the six nasopharyngeal aspirates, a respiratory virus infection was codetected in the same sample by standard diagnostic tests (immunofluorescence and virus culture) (three respiratory syncytial virus, one human metapneumovirus, and one influenza A virus), suggesting that KIPyV is not likely responsible for the symptoms that prompted nasopharyngeal sampling.

DISCUSSION

The finding of a circular DNA molecule with a genomic organization characteristic for a polyomavirus and with large

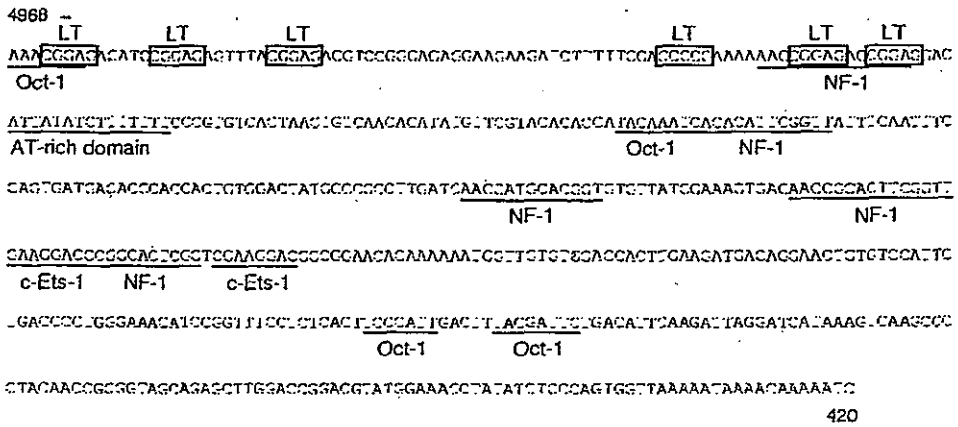

FIG. 3. The noncoding regulatory region with putative binding sites for transcription factors indicated. Putative LT antigen binding sites are boxed, and other transcription factor binding sites and the A/T-rich domain are underlined.

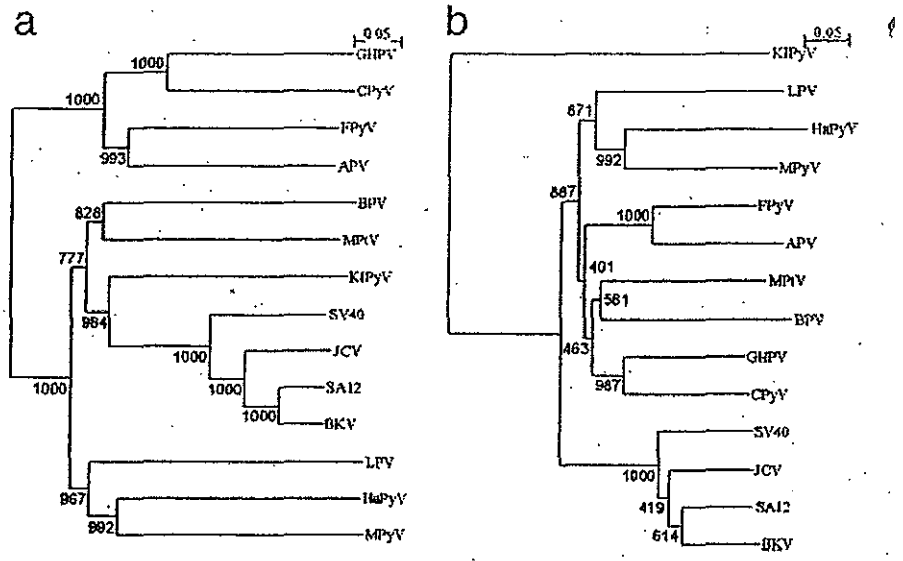

FIG. 4. Phylogenetic analysis of LT antigen amino acid sequences (a) and VP1 amino acid sequences (b). Bootstrap values are indicated at each branching point. Analysis of the ST antigen yielded a phylogenetic tree highly similar to that obtained by LT antigen analysis, and whole-genome analysis showed a similar pattern. Analysis of VP2 and VP3 yielded results highly similar to those obtained with VP1. GHPV, goose hemorrhagic polyomavirus; CPyV, crow polyomavirus; FPyV, finch polyomavirus; APV, avian polyomavirus; BPV, bovine polyomavirus; MPtV, murine pneumotropic virus; LPV, lymphotropic papovavirus; HaPyV, hamster polyomavirus.

regions of amino acid sequence homology with polyomaviruses strongly suggests that the discovered DNA molecule is indeed the genome of a previously undescribed polyomavirus. The two previously known human polyomaviruses JCV and BKV were named by the initials of the patients in whom the viruses were first identified (13, 30). This is not the current naming practice. In order to still conform to the previous nomenclature, we propose the provisional name KIPyV for the new polyomavirus. Its recovery from eight human samples confirms that it infects humans, and its partial relatedness to JCV and BKV and its prevalence in children indicate that humans are likely the natural host. However, further studies, in particular, serologic studies, are needed in order to confirm this. Intriguingly, the existence of more human polyomavirus species in addition to JCV and BKV has been suggested earlier by the finding of lymphotropic papovavirus-reactive antibodies in human sera (36). Whether this observation was indeed related to KIPyV remains to be determined. The nucleotide sequence of KIPyV has only limited similarity to that of JCV, BKV, SV40, or any other known polyomavirus. We aligned 18 published primer pairs used for the detection of JCV, BKV, or SV40 with the KIPyV sequence and found that none of them could amplify KIPyV (data not shown). It is thus highly unlikely that the presence of KIPyV would have influenced earlier studies of polyomavirus detection by PCR.

TABLE 2. Summary of results from the screening of selected human samples for KIPyV by PCR

Sample type	Source	No. of samples tested	No. (%) positive
Nasopharyngeal aspirate	Consecutive clinical samples, mainly respiratory tract disease	637	6 (1)
Urine	HSCT recipients	150	0 (0)
Serum	HSCT recipients	33	0 (0)
Whole blood	Blood donors	192	0 (0)
Feces	Consecutive clinical samples, mainly gastroenteritis	192	1 (0.5)
Isolated frozen leukocytes	Samples sent for CMV[a] diagnosis, mainly from immunosuppressed patients	96	0 (0)

[a] CMV, cytomegalovirus.

Phylogenetic analysis of the complete genome revealed that KIPyV is clearly separate from all other known polyomaviruses. When the early and late genes were analyzed separately, disparate results were obtained. While the early genes group with JCV, BKV, SV40, and SA12, the late genes form an outlier to the entire polyomavirus family. A possible explanation for this could be that the virus once emerged by recombination of two phylogenetically distant viruses, each contributing half of the genome. Alternatively, the early region may simply be more conserved because of more-rigid functional constraints while the late genes have diverged more rapidly and become very distant from those of its relatives. It is possible that future discoveries of additional polyomavirus species, e.g., in other primates, could make the phylogenetic tree more complete and provide additional clues to the evolution of KIPyV. Several new members of the polyomavirus family besides KIPyV have been discovered in the last few years (18, 19). The unique late region of KIPyV indicates that it may be the first discovered member of a new subfamily of polyomaviruses.

For assignment of nucleotide numbers, two different systems are in use for polyomaviruses. Either the nucleotide adjacent to the start codon of the T antigens, i.e., the first codon in the regulatory region, or a nucleotide in the origin of replication is considered nucleotide 1. The numbering we selected for KIPyV begins within the presumed origin and proceeds clockwise through the late region, as has been done for most pri-

mate polyomaviruses, such as JCV, SV40, SA12, and some strains of BKV.

On the basis of the ORF analysis, KIPyV is expected to express VP1 to VP3 and the ST and LT antigens, while the MT antigen and the Agno protein are both missing. The absence of the MT antigen is not surprising, since most polyomaviruses, the primate polyomaviruses included, lack expression of this particular protein. The lack of an ORF for an Agno protein is more interesting, since this protein is expressed by JCV, BKV, SV40, and SA12. The functional implications of this are unclear, since the function of the Agno protein remains to be fully elucidated. However, definitive conclusions about protein expression require further experimental evidence, e.g., in the form of mRNA analysis data.

The previously known primate polyomaviruses are generally not considered to be agents of respiratory tract disease. JCV and BKV have nevertheless been detected in human tonsil tissue, and a respiratory route of transmission of polyomaviruses has been hypothesized (9, 15, 28). BKV has also been found in the feces of children (35). The finding of KIPyV in nasopharyngeal aspirates and feces is consistent with these observations. However, the findings provide few clues to replication or latency sites or to possible disease caused by the virus. The screening of 1,300 clinical samples still provided important data. First, the cloning of a virus infecting humans was confirmed, since KIPyV genomes could be recovered from multiple individuals and since the isolates showed sequence variation. Second, the virus was detected in different age groups. Third, a concomitant finding of a recognized respiratory tract pathogen in most positive persons indicated that KIPyV was likely not the virus responsible for the respiratory tract symptoms.

The prevalence of KIPyV in humans remains unknown. Development of an antibody assay and/or finding relevant material for detecting latent virus is necessary for improved estimates. The findings obtained with nasopharyngeal aspirates suggest that the KIPyV prevalence is at least 1% in our study population. The absence of KIPyV in urine samples suggests that the biology and/or prevalence of KIPyV in kidneys differ significantly from those of JCV and BKV.

The cell type tropism and host range of polyomaviruses stem from both their regulatory regions and the receptor binding characteristics. The existence of multiple predicted c-Ets-1 transcription factor binding sites prompted us to investigate whether KIPyV may possibly replicate in lymphocytes in accordance with lymphotropic papovavirus, which harbors three putative binding sites for this transcription factor (38). This hypothesis is consistent with KIPyV being detected in the nasopharynx during an inflammatory process due to infection by a respiratory virus. However, studies of whole blood of healthy subjects or purified peripheral blood leukocytes of immunosuppressed patients did not support this hypothesis. On the other hand, given the 1% recovery rate in the respiratory tract samples, sample numbers may still be too small for definite conclusions. It must also be recognized that KIPyV is as yet only known by its genome sequence as detected by PCR. The virus has not been replicated in vitro, and no assay for detection of antibodies is available. Such experiments will be important in the further characterization of this virus.

A few newly discovered viruses have still not been associated with disease (20, 29). Nonetheless, the majority of known human viruses are pathogenic in one situation or another and any newly discovered virus must therefore be considered a likely pathogen. A problem with persisting viruses is that they are often discovered out of their symptomatic context, so that establishing their association with a particular disease may require extensive investigation. Historically, this has been the case for hepatitis B virus, Epstein-Barr virus, and parvovirus B19 (5, 8, 11). JCV and BKV are also very prevalent viruses that cause disease only under rare circumstances. Searching for a disease associated with KIPyV will be challenging but may have important medical implications. Primary candidate diseases could include unclear infectious complications in immunocompromised individuals and different types of cancer. Whether JCV, BKV, and SV40 can contribute to tumor development in humans is still a matter of debate, and one could assume that KIPyV will be subject to the same discussion. There are putative binding sites for p53, as well as the Rb family of tumor suppressor proteins, in the LT antigen of KIPyV, which indicates that a role for this virus in tumorigenesis cannot be excluded.

This study reinforces the notion that many human viruses have eluded detection despite more than 100 years of research in virology. Since viruses are likely pathogens, their identification remains an urgent scientific task. This study further illustrates how molecular virus screening of respiratory tract samples can be applied for discovering unknown viruses of different types, and not only agents of respiratory tract disease, thus making it a suitable approach for a "human virome project".

ACKNOWLEDGMENTS

We thank Inga Karlsson, Cecilia Lindau, Hamid Darban, and Daryoush Rahmani for excellent technical assistance and Lisbeth Barkholt and Fredrik Boström for providing samples.

This study was supported by the Torsten and Ragnar Söderberg Foundation, the Swedish Cancer Foundation, Nanna Svartz' Fund, the Gustav Vth Jubilee Society, and the Swedish Society for Clinical Microbiology. T.A. is a fellow of the Swedish Research Council.

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識別番号・報告回数			報告日	第一報入手日 2007 年 5 月 21 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	人ハプトグロビン	研究報告の公表状況	PLoS Pathogens 2007; 3(5 E64): 1-10	公表国 アメリカ，オーストラリア
販売名（企業名）	ハプトグロビン注－ヨシトミ(ベネシス)			

研究報告の概要

我々は、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ポリオーマウイルスの同定について報告する。このウイルスは、最初は肺炎と診断されたオーストラリアの 3 歳の子供の鼻咽頭吸引物中に検出された。
我々は、5,229 の塩基対からなるウイルスの全ての遺伝子を配列決定し、その後 WU ウイルスと名付け、このウイルスがポリオーマウイルス科に特異的な遺伝的特徴を有していることを見出した。
系統樹分析から、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。オーストラリアのブリスベン、クイーンズランド、及び米国のセントルイス、ミズーリにおける急性呼吸器感染症に罹った 2,135 人の患者を、WU ウイルスに特異的な PCR プライマーを用いてスクリーニングしたところ、WU ウイルスを含んだ 43 の検体が同定された。2 つの大陸でこのウイルスの症例が多数存在することから、このウイルスがヒトの集団において地理的に拡大していることが示唆されるとともに、WU ウイルスがヒトの病原体である可能性が高まった。

報告企業の意見

急性呼吸器感染症に罹った患者から新規のポリオーマウイルス（WU ウイルス）を同定したとの報告である。なお、このウイルスと近似のウイルスの発見報告を BENE2007-014 で報告する。
ポリオーマウイルスは、直径 40nm のエンベーロープを有しない DNA ウイルスである。万一原料血漿にポリオーマウイルスがが混入したとしても、EMC 及び CPV 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

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-Ⅰ抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理（ナノフィルトレーション）を施しているが、投与に際しては、次の点に十分注意すること。

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Identification of a Novel Polyomavirus from Patients with Acute Respiratory Tract Infections

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We report the identification of a novel polyomavirus present in respiratory secretions from human patients with symptoms of acute respiratory tract infection. The virus was initially detected in a nasopharyngeal aspirate from a 3-year-old child from Australia diagnosed with pneumonia. A random library was generated from nucleic acids extracted from the nasopharyngeal aspirate and analyzed by high throughput DNA sequencing. Multiple DNA fragments were cloned that possessed limited homology to known polyomaviruses. We subsequently sequenced the entire virus genome of 5,229 bp, henceforth referred to as WU virus, and found it to have genomic features characteristic of the family Polyomaviridae. The genome was predicted to encode small T antigen, large T antigen, and three capsid proteins: VP1, VP2, and VP3. Phylogenetic analysis clearly revealed that the WU virus was divergent from all known polyomaviruses. Screening of 2,135 patients with acute respiratory tract infections in Brisbane, Queensland, Australia, and St. Louis, Missouri, United States, using WU virus–specific PCR primers resulted in the detection of 43 additional specimens that contained WU virus. The presence of multiple instances of the virus in two continents suggests that this virus is geographically widespread in the human population and raises the possibility that the WU virus may be a human pathogen.

Citation: Gaynor AM, Nissen MD, Whiley DM, Mackay IM, Lambert SB, et al. (2007) Identification of a novel polyomavirus from patients with acute respiratory tract infections. PLoS Pathog 3(5): e64. doi:10.1371/journal.ppat.0030064

Introduction

Viral infections of the respiratory tract are responsible for significant mortality and morbidity worldwide [1]. Despite extensive studies in the past decades that have identified a number of etiologic agents, including rhinoviruses, coronaviruses, influenzaviruses, parainfluenzaviruses, respiratory syncytial virus, and adenoviruses, approximately 30% of all cases cannot be attributed to these agents, suggesting that additional respiratory pathogens are likely to exist [2]. In fact, since 2001, six previously undescribed viruses have been identified by analysis of clinical specimens from the human respiratory tract: human metapneumovirus [3], SARS coronavirus [4], coronavirus NL63 [5], coronavirus HKU1 [6], human bocavirus [7], and the recently described KI virus [8]. In some instances, new molecular methods such as VIDISCA [5], pan-viral DNA microarrays [9], and high throughput sequencing [7,8] have played key roles in the identification of these agents. The advent of these new technologies has greatly stimulated efforts to identify novel viruses in the respiratory tract and in other human disease states.

Viruses in the family Polyomaviridae possess double-stranded DNA genomes and infect a variety of avian, rodent, and primate species. To date, two polyomaviruses, BK virus and JC virus, have been unambiguously described as human pathogens. BK and JC viruses are ubiquitous worldwide, and in adult populations, seroprevalence rates approaching 75% and 100%, respectively, have been reported [10]. Although human polyomaviruses have been suggested to utilize a respiratory route of transmission, detection of BK and JC polyomavirus nucleic acids in the respiratory tract has rarely been reported [11,12]. Infection with these two viruses is predominantly asymptomatic, although in the context of immunosuppression a number of syndromes have been clearly linked to these viruses. JC virus causes primary multifocal leukoencephalopathy, while BK virus has been associated with a variety of renal and urinary tract disorders, most importantly tubular nephritis, which can lead to allograft failure in renal transplant recipients and hemorrhagic cystitis in hematopoietic stem cell transplant recipients [13]. These viruses are believed to persist in a latent phase primarily in the kidney and can periodically undergo reactivation. Excretion of BK and JC viruses in urine has been reported in up to 20% of the general population [14,15].

Editor: Donald E. Ganem, University of California San Francisco, United States of America

Received February 9, 2007; Accepted March 19, 2007; Published May 4, 2007

Abbreviations: bp, base pair; LTAg, large T antigen; NPA, nasopharyngeal aspirate; STAg, small T antigen

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Author Summary

We have identified a novel virus, referred to as WU virus, in the family Polyomaviridae by screening of human respiratory secretions. Two human polyomaviruses, BK and JC, were identified in 1971 and infect the majority of humans around the world. These two viruses are closely related to each other and are both pathogenic in immunocompromised individuals. Earlier this year, a third polyomavirus, KI, was described in human clinical specimens although its pathogenicity and prevalence in humans has not yet been established. The discovery of WU virus brings the number of polyomaviruses detected in humans to four. WU differs from BK and JC significantly in its genome sequence and in its predicted protein sequences, suggesting that it likely has unique biological properties. This discovery raises many questions for further investigations such as: is WU virus a human pathogen? If so, what kind of disease does it cause? Where in the body does WU virus reside? At what age does infection typically occur? Perhaps most importantly, there are likely to be many more as yet undetected viruses infecting the human body.

Besides JC and BK virus, a very recent report has described a novel polyomavirus, KI, detected in human respiratory secretions and stool [8]. However, the pathogenicity and prevalence of this virus has not yet been established. In addition, in the late 1950s, ~100 million people in the United States, and many more worldwide, may have been exposed to SV40, a polyomavirus that naturally infects rhesus monkeys via contaminated polio vaccines, leading to widespread debate about whether or not SV40 is capable of sustained infection and replication cycles in humans [16].

Much of the interest in polyomaviruses and SV40 in particular derives from the transforming properties carried by the early transcriptional region of the viral genome that encodes for the small T antigen (STAg) and and large T antigen (LTAg). T antigen is capable of binding both p53 and Rb proteins and interfering with their tumor suppressor functions. The early region alone is sufficient to transform established primary rodent cell lines [17] and in concert with telomerase and *ras* transforms primary human cells [18]. This has lead to controversy over whether any human tumors are associated with SV40 infection [19].

We describe the identification and characterization of a novel polyomavirus initially detected by high throughput sequencing of respiratory secretions from a patient suffering acute respiratory disease of unknown etiology. The virus was detected in the respiratory secretions from an additional 43 patients in two continents, and the complete genomes of multiple isolates were sequenced.

Results

Shotgun Sequencing of Respiratory Secretion

A nasopharyngeal aspirate (NPA) from a 3-year-old patient admitted to the pediatric ward of the Royal Children's Hospital in Brisbane with pneumonia was collected in October 2003. The patient had no other remarkable clinical traits other than the respiratory features of pneumonia. Testing of nucleic acid extracted from the NPA using a panel of 17 PCR assays for known respiratory viruses as described [20] yielded negative results. Total nucleic acid from the NPA was randomly amplified and cloned as described previously [9]. One 384-well plate of clones was sequenced using a universal M13 primer, and the resulting sequence reads were analyzed as described in Materials and Methods. Of the 384 reads, there were 37 poor quality sequences that were rejected from further analysis, 327 human sequences, six bacterial sequences, six viral sequences, and eight sequences of unknown origin that could not be classified. The bacterial sequences had greater than 97% nucleotide identity to known bacterial species, including *Haemophilus influenzae* (three reads), *Streptococcus pneumoniae*, *Corynebacterium pseudodiphthericum*, and *Leifsonia xyli* (unpublished data). Upon further examination, the six viral reads were collapsed into three unique regions, each of which possessed only limited homology to known polyomavirus proteins (sequences available in Figure S1). The highest scoring BLASTx hits for each of these three contigs possessed 35%, 50%, and 34% amino acid identity to JC virus STAg, BK virus LTAg, and SV40 VP1, respectively. At the time these experiments were performed, the KI virus genome had not yet been published. Subsequent analysis revealed amino acid identities of 66%, 65%, and 69% to KI virus for the three contigs. Furthermore, three of the eight previously unclassified sequence reads were determined to have between 58%–84% amino acid identity to KI virus VP1 and VP2 proteins by BLASTx analysis. Based on the limited sequence homology to known viruses, we tentatively assigned the name WU to the unknown polyomavirus.

Complete Genome Sequencing and Genome Analysis

The complete genome of WU was sequenced to 3× coverage using cloned fragments of the viral genome generated by a series of PCR primers. Analysis of the DNA sequence revealed genomic features characteristic of polyomaviruses. First, the WU genome size of 5,229 base pairs (bp) was quite comparable to those of the primate polyomaviruses BK (5,153 bp), JC (5,130 bp), and SV40 (5,243 bp). In addition, the overall GC content of the WU genome was 39%, which is quite similar to the GC content of BK (39%), JC (40%), and SV40 (40%). The genome organization included an early region coding on one strand for STAg and LTAg, and a late region coding on the opposite strand for the capsid proteins VP1, VP2, and VP3 (Figure 1). These two regions were separated by a regulatory region that contained typical polyomavirus features. The regulatory region contained an AT-rich region on the late side of the putative replication origin. Three repeats of the consensus pentanucleotide LTAg binding site GAGGC were present, as was one copy of the non-consensus LTAg binding site TAGGC. While most polyomaviruses contain four copies of the consensus, baboon polyomavirus (simian agent 12) is a primate polyomavirus that contains only three copies of the canonical binding sequence and one non-consensus binding site [21]. Unusual features in the WU regulatory region included the presence of two partially overlapping LTAg binding sites and slightly variant spacing between the LTAg binding sites as compared to SV40, BK, and JC (Figure S2).

In the early region, an unspliced open reading frame of 194 amino acids was detected that possibly encodes for the STAg. As the paradigm in other polyomaviruses is that STAg is expressed from a spliced message, analysis of potential splice sites revealed the presence of a putative splice donor sequence just one nucleotide 5′ of the initially predicted

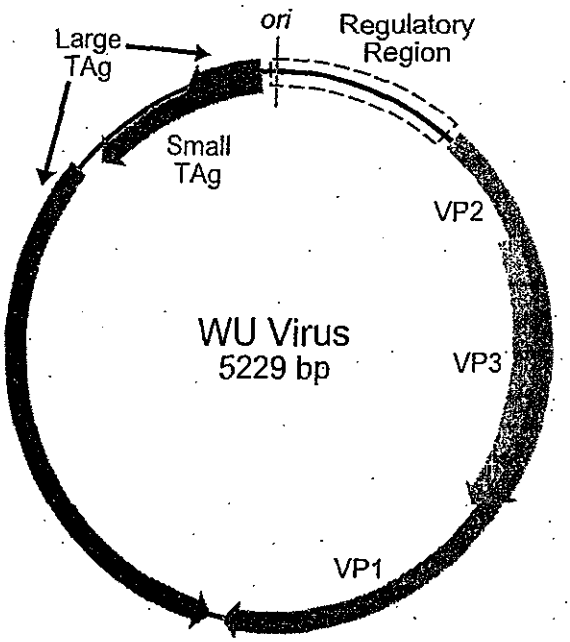

Figure 1. Schematic of WU Virus Genome Organization
ori, origin of replication.
doi:10.1371/journal.ppat.0030064.g001

stop codon. Splicing to a downstream putative splice acceptor site would excise an intron of 70 nucleotides and generate a slightly larger STAg of 217 amino acids (Figure S3). While the precise carboxyl terminus of the WU STAg has not yet been experimentally verified, sequence analysis revealed the presence of a highly conserved cysteine-rich motif, $CX_5CX_{7-8}CXCX_2CX_{21-22}CSCX_2CX_3WF$, that was present in both of the predicted isoforms of WU STAg. This motif, which is present in all STAgs, was perfectly conserved in WU virus with the exception of the initial cysteine residue.

In all polyomaviruses, the initial ~80 amino acids of the N-terminus of the STAg and LTAg are identical; the LTAg is generated by alternative splicing of the early mRNA transcript. In WU virus, a conserved splice donor site was identified immediately after amino acid 84 of the early open reading frame. The position of the splice site is similar to that found in SV40, BK, and JC virus, which occur after amino acids 82, 81, and 81, respectively. Splicing to a conserved splice acceptor site would generate a predicted protein of 648 amino acids (Table 1). The predicted WU virus LTAg contained conserved features common to T antigens, including a DnaJ domain in the N terminus with the highly conserved hexapeptide motif HPDKGG; the LxCxE motif necessary for binding Rb; a canonical DNA binding domain; a zinc finger region; and conserved motifs GPXXXGKT and GXXXVNLE in the ATPase-p53 binding domain [22].

Based on comparative sequence analysis of LTAgs, the polyomaviruses are classified into two subclasses: a primate-like group exemplified by SV40, and a mouse polyoma-like group exemplified by murine polyoma virus [22]. Using these criteria, the T antigen of WU appeared to more closely resemble the mouse polyoma-like class of virus than the primate class. First, the mouse polyoma-like viruses have insertions of varying length after amino acids 66 and 113 of SV40 as compared to the primate class. In the amino terminal domain of the WU virus LTAg, multiple sequence alignment revealed the presence of a two-amino acid and a ten-amino acid insertion at these two loci, respectively. Furthermore, the primate-like class typically contains an extension of the carboxyl terminus termed the host range domain that is absent in the mouse polyoma-like class. In contrast to SV40, BK, JC, and baboon polyomavirus, WU virus did not appear to encode a carboxyl terminal extension (Figure S4).

In addition to encoding LTAg and STAg, murine and hamster polyomaviruses utilize alternative splicing to generate an intermediate-sized protein referred to as middle T antigen. The WU virus early region was scanned for splicing motifs similar to known murine and hamster polyomavirus splice donor and acceptor sequences, but no obvious combination of splice sites was detected that would yield a middle T antigen sequence in the size range of known middle T antigens. In addition, SV40, JC, BK, and baboon polyomavirus all encode a fourth late protein termed the agnoprotein. There was no open reading frame present in WU with any detectable homology to the known agnoproteins. Thus, our sequence analysis suggests that neither middle T antigen nor agnoprotein are encoded by WU virus, although it is possible that the sequences have diverged beyond our ability to recognize the appropriate splice sites or protein products.

Phylogenetic Analysis

Multiple sequence alignments of the predicted STAg, LTAg, VP1, and VP2 open reading frames revealed that WU

Table 1. Homology of Predicted WU Proteins

Gene	Predicted Size (aa)	% Amino Acid Identity[a] to:				
		KI	JC	BK	SV40	MuPy[c]
STAg	194[b]	68	40	41	38	23
LTAg	648	70	48	49	49	32
VP1	369	65	27	28	28	25
VP2	415	71	16	17	17	12
VP3	272	64	15	15	16	11

[a]Calculated using BioEdit.
[b]The unspliced form was used to calculate % identity.
[c]MuPy, murine polyomavirus.
doi:10.1371/journal.ppat.0030064.t001

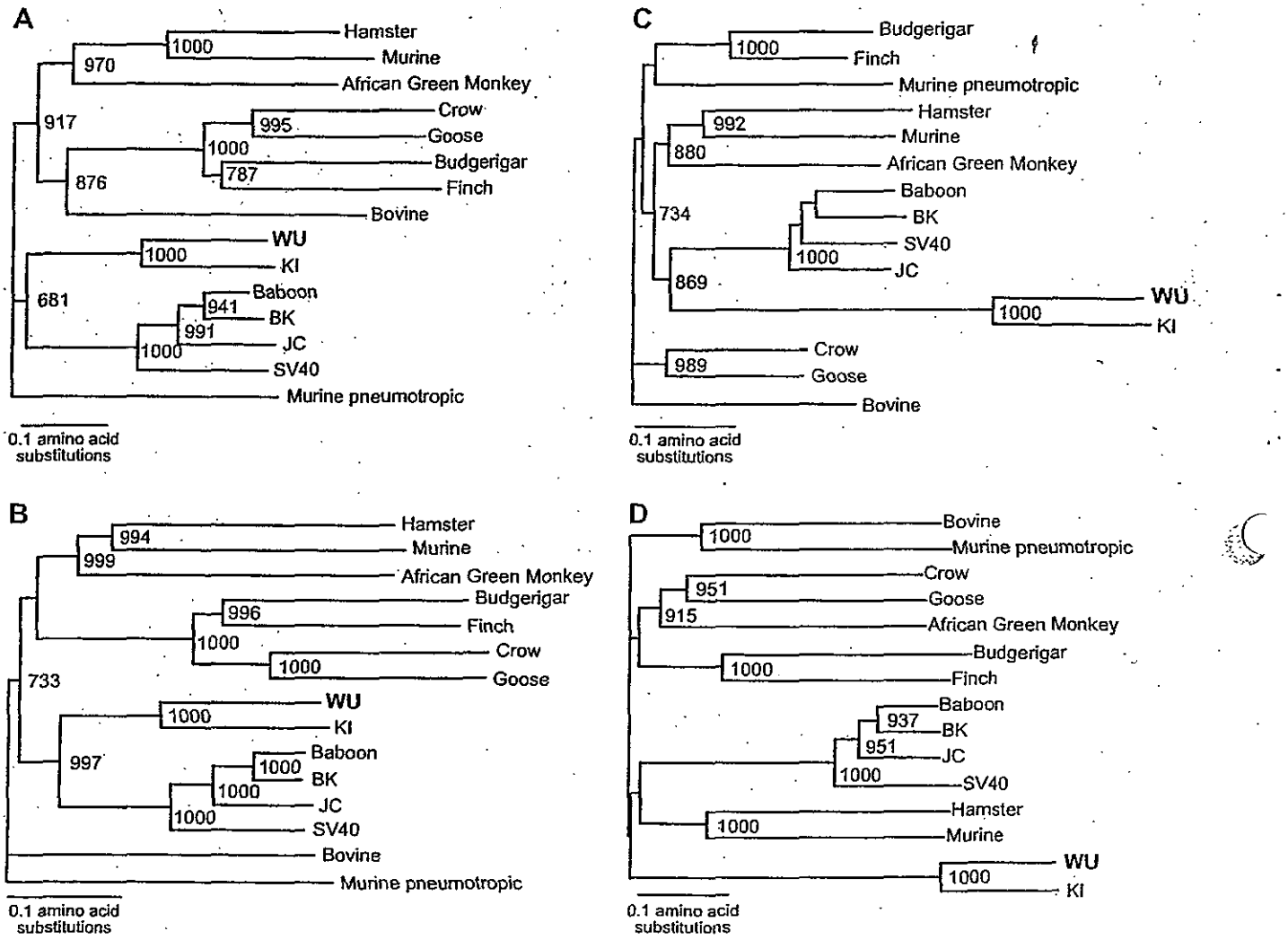

Figure 2. Phylogenetic Analysis of WU Virus

Amino acid–based phylogenetic trees were generated using the neighbor-joining method with 1,000 bootstrap replicates. Significant bootstrap values are shown. A) STAg; B) LTAg; C) VP1; D) VP2.

doi:10.1371/journal.ppat.0030064.g002

virus was clearly a novel virus that is most closely related to KI virus (Figure 2). Neighbor-joining analysis suggested that these two viruses appear to form a new subclass of polyomaviruses. In the early region and VP1 protein, the WU/KI branch was most closely related to the known primate polyomaviruses BK, SV40, JC, and baboon polyomavirus (Figure 2A–2C). Finally, the VP2 open reading frame was so divergent that its evolutionary relationship to other polyomaviruses aside from KI could not be reliably established (Figure 2D). Analysis of the VP3 amino acid sequence, which is completely contained within VP2, gave similar results as VP2 (unpublished data).

Prevalence of WU

PCR primers were designed to specifically amplify WU. The initial screen used primers targeting the VP2 region, which possessed less than 20% amino acid homology to JC and BK virus to minimize the possibility of cross reactivity with the known human polyomaviruses. Empirical testing of the primers on samples known to contain BK and JC confirmed that the primers did not cross react with either of these genomes (unpublished data). Positives in the initial screen for WU virus were sequenced and then further confirmed by a second PCR reaction using primers targeting the 3′ end of the WU virus LTAg coding sequence. All 43 positive samples in the initial screen were confirmed using the second pair of PCR primers. A subset of samples that tested negative in the initial screen was also tested with the second PCR primer pair, and none of those samples were positive.

Brisbane, Queensland, Australia, Cohort

In order to assess the prevalence of WU polyomavirus, a cohort of 1,245 respiratory specimens collected in 2003 in Brisbane was examined. Thirty-seven out of the 1,245 (3.0%) samples tested were positive for the virus (Table 2). In this cohort, patients that tested positive ranged in age from 4 months to 53 years. The vast majority of the patients (33/37) were age 3 and under. In 12 patients with clear clinical evidence of respiratory tract infection, WU was the sole virus detected. Strikingly, in 25 of the 37 positive samples, one or

Table 2. Patients Positive for WU Virus

ID	Age (Years)	Sex	Sample Type	Clinical Findings	Viral Co-Infection
WU[a]	3	M	NPA	Pneumonia	
S1	51	M	BAL	Unexplained respiratory failure, ventilated	Herpes simplex
S2	3	M	NPS	Neuroblastoma	Metapneumo
S3	0.3	M	NPS	URTI	Rhino
S4	2	M	NPS	Febrile respiratory infection with patchy pulmonary infiltrates	Influenza B
S5	0.4	F	NPS	URTI	Adeno
S6	19	F	NPS	Influenza-like illness, reactive airways disease, pregnant	Influenza B
B1	53	M	BAL	LRTI, Wegner granulomatosis	
B2	0.9	M	NPA	Bronchiolitis	
B3	43	M	BW	HIV, Kaposi sarcoma	Epstein-Barr
B4	2	M	NPA	LRTI, cystic fibrosis	
B5	2	M	NPA	LRTI, post bone marrow transplant	Respiratory syncytial virus
B6	1	F	NPA	Gastroenteritis	Rhino
B7	0.9	M	NPA	Bronchiolitis	Rhino
B8	0.8	M	NPA	Bronchiolitis	Metapneumo, rhino
B9	6	M	NPA	LRTI, febrile neutropaenia, ALL	Metapneumo
B10	1	M	NPA	URTI, gastroenteritis	
B11	2	M	NPA	Pneumonia	
B12	2	F	NPA	URTI	Bocavirus, entero
B13	2	M	NPA	LRTI, cerebral palsy	
B14	1	M	NPA	URTI	
B15	2	F	NPA	URTI	Influenza A
B16	2	F	NPA	Bronchiolitis	Rhino
B17	0.6	M	NPA	Bronchiolitis	Rhino
B18	2	M	NPA	LRTI	Bocavirus
B19	0.6	M	NPA	URTI, gastroenteritis	Rhino
B20	1	M	NPA	URTI	Bocavirus
B21	0.6	M	NPA	Bronchiolitis	
B22	0.3	M	NPA	URTI	Rhino
B23	0.6	M	NPA	URTI	Rhino
B24	1	F	NPA	URTI, febrile convulsion	Adeno, rhino, bocavirus
B25	0.8	M	NPA	Bronchiolitis	
B26	3	F	NPA	URTI	
B27	6	F	NPA	URTI, post bone marrow transplant	
B28	2	M	NPA	Infective exacerbation of bronchiectasis	Entero
B29	1	F	NPA	LRTI	
B30	0.3	M	NPA	URTI	Rhino, bocavirus
B31	0.6	M	NPA	Bronchiolitis	Rhino
B32	3	F	NPA	URTI	Bocavirus
B33	1	M	NPA	URTI	Rhino, bocavirus
B34	0.8	M	NPA	Bronchiolitis	Bocavirus, parainfluenza 3
B35	2	F	NPA	LRTI	Rhino, bocavirus
B36	0.9	M	NPA	LRTI, ETT, ventilated	Bocavirus
B37	2	M	NPA	Croup	Rhino

[a]Original case.

ALL, acute lymphoblastic leukemia; BAL, bronchoalveolar lavage; BW, bronchial washings; ETT, endotracheal tube in place; LRTI, lower respiratory tract infection; NPS, nasopharyngeal swab; URTI, upper respiratory tract infection.

doi:10.1371/journal.ppat.0030064.t002

more additional respiratory viruses were also detected. The most common co-infections were with rhinovirus (15 cases) and human bocavirus (ten cases). Furthermore, in one sample, a total of four viruses (WU, bocavirus, rhinovirus, and adenovirus) were detected, and in six other samples, a total of three viruses were detected (Table 2).

St. Louis, Missouri, United States, Cohorts

In addition, we examined two cohorts of patients from St. Louis, Missouri, United States. In one set of upper respiratory specimens collected in 2006, five out of 410 were positive for WU virus in the PCR assay. In addition, 480 bronchoalveolar lavage samples from patients (mostly adults) with severe acute respiratory illness were tested, yielding one positive. Of the positive samples, all six were co-infected with other viruses (Table 2). The age range of the positive cases varied from 4 months to 51 years.

Strain Variants

To assess the sequence variation within different isolates, we analyzed the 250-bp region encompassed by the initial screening primers for all 43 cases (Figure 3). Several divergent strains were detected, including one sample that had five mutations (2%) within this region. In another case, a 12-bp deletion was observed. The fact that many isolates were identical in sequence was not surprising, given the relatively short length of the amplicon and the double-stranded DNA nature of the genome. In addition, we sequenced the complete genome of five additional isolates from five independent patients. Unfortunately, efforts to completely

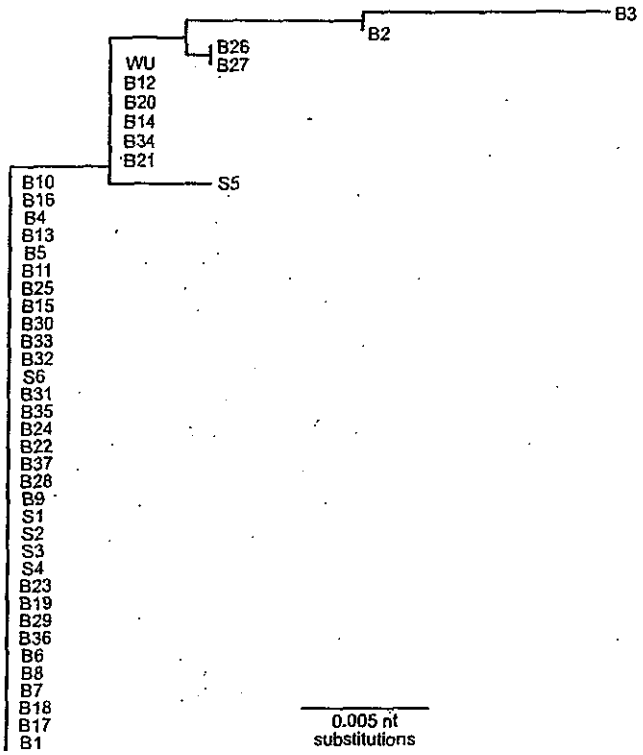

Figure 3. Strain Variation of WU virus

A 250-bp fragment of the VP2 gene was aligned using ClustalX. WU indicates the original case, and strain designations correspond to patients as listed in Table 2.

doi:10.1371/journal.ppat.0030064.g003

sequence the two most divergent isolates (based on the 250-bp sequence, B2 and B3) have been unsuccessful, presumably due to low viral titers in these samples. All six complete genomes were 5,229 bp in size, and overall, there was between 0.08% and 0.23% sequence variation from sample to sample, well above that expected from Taq PCR, ruling out the possibility that the additional positives were artifacts of PCR contamination. Moreover, the majority of the observed mutations were synonymous substitutions or in non-coding regions, lending further support to the argument that these were authentic strain variants. For JC virus, the reported intratype sequence variation is of a similar magnitude, ranging between 0.1% and 0.5% [23].

Screening of Urine

Because BK and JC virus are frequently excreted in urine, we examined urine samples from patient cohorts in both St. Louis and Brisbane for the presence of WU virus by PCR. In the St. Louis cohort, urine samples from 200 adult patients participating in a study of polyomavirus infections in kidney transplant recipients were tested [24]. For most patients, samples were tested at three time points: prior to transplant, 1 mo post transplant, and 4 mo post transplant, although for some patients the pre-transplant specimen was not available. Zero out of 501 samples tested were positive for the WU polyomavirus. As a control, using previously validated BK primers, we were able to amplify BK virus in a subset of these urine samples, confirming the integrity of the specimens themselves (unpublished data). Similarly, from the Brisbane cohort, none of the 226 urine samples tested were positive for WU virus.

Discussion

We used a high throughput sequencing strategy to search for novel agents that were present in respiratory tract infections of unknown etiology. The focus of this study was on individual clinical specimens that still lacked a diagnosis after analysis with an extensive panel of diagnostic assays for known respiratory viruses. In one such patient sample, novel sequences with limited homology to known polyomaviruses were detected. Complete genome sequencing and phylogenetic analysis revealed that the new virus clearly had the genomic organization typical of polyomaviruses but was divergent from all previously described polyomaviruses. In keeping with the two-letter virus names for human polyomaviruses, we have named this novel polyomavirus WU virus [25,26]. Overall, the predicted amino acid sequences of WU virus proteins were most similar to the newly described KI virus (Table 1). Outside of KI, WU shared only ~15%–49% identity to its closest relatives (Table 1).

Detailed analysis of the viral DNA sequence and genomic organization confirmed the novelty of WU virus. At all loci, WU virus was most similar to KI virus, but the degree of divergence between WU and KI was greater than the divergence between SV40 and BK, indicating that WU and KI are clearly distinct viruses (Figure 2). Based on the phylogenetic analysis, it appears that WU and KI define a novel branch within the Polyomaviridae family (Figure 2). Relative to the established polyomaviruses, some analyses suggested that the WU/KI branch might be more closely related to the primate polyomaviruses, while other features of the WU genome suggested that it might be more similar to murine polyomavirus. For example, neighbor-joining phylogenetic analysis suggested that the predicted STAg, LTAg, and VP1 open reading frames of both KI and WU were most closely related to SV40, JC, BK, and baboon polyomaviruses. Analysis of the VP2/VP3 region was more equivocal, as the proteins were too divergent to reliably assess. The apparent absence of the C-terminal "host range" domain in the LTAg and the agnoprotein open reading frame, both of which are present in the known primate polyomaviruses, suggested that WU virus was more similar to murine polyomavirus than the primate polyomaviruses by these criteria. While the evolutionary history of this virus is not clear at the moment, the totality of the analysis indicates that WU is clearly a unique virus.

We detected WU in 37 out of 1,245 (3.0%) patient specimens in Brisbane (excluding the original case) and in six out of 890 (0.7%) patient specimens tested in St. Louis. As the positive specimens were all collected from 2003 through 2006, it appears that WU is currently circulating, and its presence in both North America and Australia suggests that the virus is geographically widespread in the human population. The age range of patients that tested positive for WU virus spanned from 4 months to 53 years. The majority (86%) of the cases were found in children 3 years of age and under. Of the four positive specimens from adult patients (S1, S6, B1, and B3 in Table 2), three clearly had altered immune status. One patient was HIV-positive, one was immunosuppressed due to treatment for Wegener granulo-

matosis, and one was pregnant. The fourth adult patient (S1), while not obviously immunosuppressed, also suffered from liver cirrhosis, hypertension, type 2 diabetes, and co-infection with herpes simplex virus, and required mechanical ventilation. In addition, there were two other positive patients older than 3 years of age: a 6-year-old child who had previously been a bone marrow transplant recipient (Table 2, B27) and a 6-year-old child diagnosed with acute lymphoblastic leukemia (Table 2, B9). While preliminary, the age distribution of the positive cases in this study combined with the established paradigms for BK and JC virus suggest a model where acute infection with WU virus may occur relatively early in life and result in a latent infection. Immunosuppression or other insults such as viral infection could then lead to reactivation of WU virus in older individuals.

The patients who yielded positive specimens suffered from a wide range of respiratory syndromes, including bronchiolitis, croup, and pneumonia as well as other clinical maladies (Table 2). Detection of WU virus sequences in these patients is merely the first step in assessing the potential etiologic role of WU virus in acute respiratory tract disease. It is not yet known whether WU is infectious or whether it is capable of replication in the respiratory tract. One possibility is that WU is not involved at all in respiratory disease, but rather is simply transmitted by the respiratory route. The human polyomaviruses BK and JC are hypothesized to be transmitted by the respiratory route before taking up residency primarily in the kidneys. Latency in the kidneys of BK and JC is believed to be the reason that both viruses are excreted in the urine of up to 20% of asymptomatic individuals [14,15]. In this study, using the same PCR assays that were effective in respiratory secretions, we did not detect WU in any of the 727 urine samples we tested. The lack of detection of WU virus in the urine may reflect sensitivity issues, a bias in the cohorts tested, or simply that WU is unlike BK and JC viruses and is not secreted in the urine. A similar tissue profile to that of WU virus has been reported in initial studies of KI virus [8]. Future experiments will aim to determine the tissue tropism of WU and whether any tissue reservoirs for WU virus exist.

In the literature, there is one animal polyomavirus that has been found extensively in lung tissue. Infection of suckling mice with the mouse pneumotropic polyomavirus (MPPV) causes interstitial pneumonia and significant mortality. MPPV also differs from other polyomaviruses in that besides the kidneys, it can also be detected in the lungs, liver, spleen, and blood of suckling mice [27]. Thus, there is precedence for an animal polyomavirus causing respiratory disease, suggesting at least the possibility that WU virus could be similarly pathogenic in humans.

One striking observation from these studies is the relatively high frequency of co-infection detected in the respiratory secretions: 72% overall (100% in the St. Louis cohort and 68% in the Brisbane cohort). Although more extensive studies are necessary to confirm the generality of this observation, this raises several intriguing non-mutually exclusive possibilities to consider: 1) WU may be an opportunistic pathogen; 2) WU infection may predispose or facilitate secondary infection by other respiratory viruses; and 3) WU may be a part of the endogenous viral flora that is reactivated by inflammation or some other aspect of viral infection. Recent studies of the prevalence of the newly identified human bocavirus have also reported higher levels of co-infection than previously described for other viruses found in the respiratory tract, with co-infection rates as high as 50% reported [28,29]. In addition, five of six samples positive for KI virus were reported to be co-infected with other known respiratory viruses [8]. As detection methods improve in sensitivity and more comprehensive efforts are made to examine the diversity of viruses found in the respiratory tract, a greater appreciation for the rates of dual or multi-infection is gradually emerging. For example, the use of extensive panels of PCR assays in this study revealed that one of the positive specimens was quadruply infected; adenovirus, rhinovirus, and bocavirus and WU virus were all present. Further investigations that aim to systematically define the spectrum of viruses present in the respiratory tract are clearly warranted so that the possible roles that co-infections may play in disease pathogenesis can be explored.

Extremely high sequence divergence was observed in the capsid proteins VP1 and VP2 of WU virus and KI virus as compared to the other known polyomaviruses. This divergence may reflect a different "lifestyle" for the WU/KI branch as compared to known polyomaviruses. Our data demonstrating the presence of WU in respiratory secretions and its absence in urine samples suggest that the mode of transmission or the sites of persistence of WU may be distinct from the other human polyomaviruses. As such, the structure of the virion must be optimized to enable the virus to survive dramatically distinct physiological and environmental conditions. This may partially explain the observed sequence divergence in the capsid proteins.

Another question raised by this study relates to the potential antigenic cross reactivity of the WU capsid proteins. In terms of establishing the seroprevalence of WU itself and determining whether seroconversion accompanies acute infection with WU, it will be essential to conduct these studies with consideration for potential cross reactivity to KI, BK, JC, and SV40 antibodies. In addition, it is tantalizing to speculate whether serum antibodies to WU have the potential to cross react to SV40-derived antigens, and if so, whether they may at least partially account for some of the studies that report the presence of SV40 antibodies in the human population that is too young to have suffered exposure from contaminated polio vaccination [30–32].

In conclusion, we have identified and completely sequenced the genome of a novel polyomavirus. This virus appears to be geographically widespread in the human population as evidenced by the detection of 44 distinct cases in two continents. Based on preliminary analysis, WU and KI virus share some strikingly similar properties, including their complement of genes, phylogenetic relationship, and physical sites of detection in the human body. These data suggest that WU virus and KI virus define a novel branch within the Polyomaviridae family with unexplored biology and pathogenicity. Another implication of these results is that the diversity of viruses in this family may be far greater than currently realized. Further experimentation is now underway to determine the relative pathogenicity of WU virus in humans and to understand the molecular properties of the virus. Since the T antigen of WU is predicted to have transforming properties by analogy to other polyomavirus T antigens, one question currently under investigation is whether a subset of human tumors may be associated with WU.

Materials and Methods

Clinical specimens—Respiratory secretions. Brisbane cohort. A total of 1,245 specimens (predominantly NPAs) were collected between January 1, 2003, and December 22, 2003, from patients presenting to the Royal Children's Hospital in Brisbane, Queensland, Australia, with symptoms consistent with acute lower respiratory tract infection.

St. Louis cohort #1. A total of 480 BAL specimens were tested. These included samples from a retrospective and a prospective collection. The retrospective specimens were from a sequential collection of BAL specimens submitted routinely to the Virology Laboratory at St. Louis Children's Hospital between December 2002 and August 2003 [33]. For the present study, an effort was made to select specimens from this collection from patients with acute respiratory illness, and to exclude specimens collected as routine post–lung transplant surveillance. The prospective specimens were from an ongoing study of the etiology of severe acute respiratory illness and were collected between October 2005 and October 2006. Both collections included specimens from patients of all ages, although the large majority were from adults.

St. Louis cohort #2. This collection was made up of respiratory specimens, mostly nasopharyngeal swabs, submitted for routine virologic testing to the Virology Laboratory at St. Louis Children's Hospital between September 2005 and June 2006. The majority of these specimens were from children. Of the 410 specimens in this collection, 200 were selected because they had been found to be positive by fluorescent antibody staining or culture for influenzavirus A or B, respiratory syncytial virus, parainfluenza virus, rhinovirus, or adenovirus.

Clinical specimens—Urine. Brisbane cohort. Urine specimens (226) that were submitted during 2003 to the diagnostic laboratory for routine investigation were collected. These represented a diverse mixture of donors, including those from (i) sexual health clinic ($n = 50$), (ii) pediatric clinic ($n = 52$), (iii) antenatal clinic ($n = 33$), (iv) indigenous health clinic ($n = 36$), and (v) bone marrow transplant patients ($n = 55$).

The St. Louis urine specimens were from a study of polyomaviruses in adult renal transplant recipients [24]. A total of 200 individuals were enrolled in the study between December 2000 and October 2002. From each patient, up to three specimens were tested, including a specimen obtained before the transplant and specimens obtained at 1 and 4 mo after transplantation.

Diagnostic testing of clinical specimens for known respiratory viruses. Brisbane cohort. Nucleic acids were extracted from 0.2 ml of each specimen using the High Pure Viral Nucleic Acid kit (Roche Diagnostics Australia, http://www.rochediagnostics.com.au) according to the manufacturer's instructions. PCR assays for 17 known respiratory viruses were performed as described [20].

St. Louis cohort. All respiratory specimens were tested originally by fluorescent antibody staining using a panel of monclonal antibodies directed against influenza A and B, respiratory syncytial, parainfluenza 1–3, and adenoviruses (Simulfluor Respiratory Screen; Chemicon, http://www.chemicon.com). Specimens that were negative were also cultured using cell culture systems that could detect the same group of viruses plus rhinoviruses, cytomegalovirus, and herpes simplex virus. Total nucleic acid extracts were purified using a Qiagen M48 instrument (http://www.qiagen.com). Nucleic acid extracts were tested for a panel of respiratory viruses using the EraGen MultiCode-PLx respiratory virus panel (EraGen Biosciences, http://www.eragen.com), a multiplex PCR assay that detects the following viruses: influenza A and B, respiratory syncytial virus A and B, parainfluenza 1–4, human meatpneumovirus, adenovirus subgroups B, C, and E, rhinoviruses, and coronaviruses OC43, 229E, and NL63.

Library construction and shotgun sequencing. Samples were prepared in the following manner for high throughput sequencing analysis. A total of 200 ul of neat NPA sample was thawed and directly treated with DNase I (Fermentas, http://www.fermentas.com) for 60 min at 37 °C. Total nucleic acid was extracted using the Masterpure Complete DNA and RNA Purification Kit (Epicentre Biotechnologies, http://www.epibio.com). Then, 100 ng of total nucleic acid was randomly amplified using the RdAB protocol exactly as described [9]. RNA in the total nucleic acid preparation was converted to cDNA by reverse transcription with primer-A (5′ GTTTCCCAGTCACGATANNNNNNNNN). Two rounds of random priming with primer-A and extension with Sequenase (United States Biochemical, http://www.usbweb.com) enabled second strand cDNA synthesis as well as random priming of DNA originally present in the total nucleic acid sample. Amplicons were then generated via 40 cycles of PCR using primer-B (5′ GTTTCCCAGTCACGATA) with a cycling profile of: 94 °C 30 s; 40 °C 30 s; 50 °C 30 s; 72 °C 60 s. The primer-B–amplified material was TOPO cloned into pCR4.0 (Invitrogen, http://www.invitrogen.com) and transformed into bacteria, and white colonies were picked into 384-well plates. DNA was purified by magnetic bead isolation and sequenced using standard Big Dye terminator (v3.1) sequencing chemistry. Reaction products were ethanol precipitated, resuspended in 25 ul of water, and loaded onto the ABI 3730xl sequencer.

Analysis of shotgun sequences. Sequences were assessed for quality using Phred [34], and reads that contained less than 50 contiguous bases with a score of phred 20 or greater were rejected. The remaining reads were analyzed in the following steps: 1) reads were aligned to the human genome using BLASTn with an e^{-10} cutoff; 2) remaining reads were aligned to a bacterial database using BLASTn with an e^{-10} cutoff; and 3) remaining reads were aligned to the viral RefSeq protein database using BLASTx with an e^{-2} cutoff [35].

Complete genome amplification and sequencing. The WU genome derived from the index case was sequenced to 3× coverage using six unique pairs of PCR primers for the amplification. Amplicons were cloned into pCR4.0 and sequenced using standard sequencing technology. All primers used for amplification and sequencing are listed in Table S1 and their positions depicted in Figure S5. Additional complete genomes were sequenced to at least 2× coverage using the same primers listed in Table S1. Completed genome sequences have been deposited into GenBank (see Supporting Information for accession numbers).

Phylogenetic analysis. Protein sequences associated with the following reference virus genomes were obtained from GenBank: BK virus, JC virus, bovine polyomavirus, SV40, baboon polyomavirus (simian agent 12), finch polyomavirus, crow polyomavirus, goose hemorrhagic polyomavirus, African green monkey polyomavirus, budgerigar fledgling polyomavirus, murine pneumotropic virus, hamster polyomavirus, and murine polyomavirus (see Supporting Information for accession numbers). For WU virus, predicted open reading frames were used. For STAg, the predicted open reading frame of 194 amino acids was used for analysis. Multiple sequence alignment was performed using ClustalX (1.83). Neighbor-joining trees were generated using 1,000 bootstrap replicates.

Nucleic acid prevalence studies. For all PCR assays, standard precautions to avoid end product contamination were taken, including the use of PCR hoods and maintaining separate areas for PCR set up and analysis. For initial screening of WU virus, PCR primers AG0044 5′ tgttacaaatagctgcaggtcaa and AG0045 5′ gctgcataatggggagtacc were used with Accuprime hot start Taq (Invitrogen) to amplify 1 ul of template using the following program: 40 cycles of 94 °C 30 s; 56 °C 30 s; 72 °C 60 s. For every 88 samples tested, seven no-template negative controls were interspersed between the actual samples. Products were visualized following electrophoresis on 1% agarose gels. The resulting 250-bp amplicon was sequenced directly in both directions using primer AG0044 and AG0045. These sequences have been deposited in GenBank (see Supporting Information for accession numbers). Secondary confirmation was performed using primers AG0048 5′ TGTTTTTCAAGTATGTTGCATCC and AG0049 5′ CACCCAAAAGACACTTAAAAGAAA that generate a 244-bp amplicon in the 3′ end of the LTAg coding region. The same cycling profile of 40 cycles of 94 °C 30 s; 56 °C 30 s; 72 °C 60 s was used. For detection of both BK and JC viruses, primers AG0068 5′ AGTCTTTAGGGTCTTCTACC and AG0069 5′ GGTGCCAACCTATGGAACAG were used with a profile of 40 cycles of 94 °C 30 s; 56 °C 30 s; 72 °C 60 s.

Supporting Information

Figure S1. Raw Sequence Data from High Throughput Screening

A) The initial six shotgun reads with homology to polyomaviruses. B) The three contigs derived from the six reads.

Found at doi:10.1371/journal.ppat.0030064.sg001 (38 KB PDF).

Figure S2. Comparison of SV40 and WU Virus Replication Origin Region

The consensus TAg binding motif is GAGGC. The known primate polyomaviruses SV40, JC, BK, and baboon polyomavirus all have four copies of the copies of the binding site oriented as shown above for SV40 (NC_001669). The first nucleotide of the third copy of the consensus TAg binding site is defined as nucleotide 1 for WU and SV40. Differences between SV40 and WU Virus are 1) one of the TAg binding sites in WU virus appears to be a non-canonical TAGGC; 2) the second and third consensus TAg binding sites in WU virus

overlap; and 3) the nucleotide spacing between the TAg binding sites in WU virus varies from the prototype SV40 as shown. Shown in blue is the polyA/T tract that is commonly found to the late side of the origin in polyomaviruses

Found at doi:10.1371/journal.ppat.0030064.sg002 (462 KB PDF).

Figure S3. Predicted Splice Sites for LTAg and STAg

A consensus LTAg donor site was detected. Splicing to the consensus downstream acceptor would generate a LTAg of 648 amino acids. For STAg, an unspliced open reading frame of 194 amino acids was identified. A predicted slice donor site was also detected that would result in excision of a 70-nucleotide intron and production of a 217-amino acid open reading frame.

Found at doi:10.1371/journal.ppat.0030064.sg003 (542 KB PDF).

Figure S4. WU Virus Lacks a Carboxyl Terminus Extension of the the LTAg

Multiple sequence alignment of WU virus LTAg with 13 other reference sequences reveal the presence of carboxyl terminus extensions in baboon polyoma, BK, JC, and SV40. WU virus does not appear to encode such a region.

Found at doi:10.1371/journal.ppat.0030064.sg004 (5.4 MB PDF).

Figure S5. Map of Primers and Sequence Reads

Locations of original shotgun reads are depicted as indicated. Locations of all sequencing primers are mapped to the complete genome. Primers used for amplification are shown in red.

Found at doi:10.1371/journal.ppat.0030064.sg005 (551 KB PDF).

Table S1. Primers Used for Amplification and Sequencing of WU Virus

Found at doi:10.1371/journal.ppat.0030064.st001 (35 KB PDF).

Accession Numbers

The GenBank (http://www.ncbi.nlm.nih.gov/Genbank) protein sequences used in this paper are as follows:

LTAg: African green monkey (NP_848008); baboon polyomavirus 1 (YP_406555); BK (YP_717940); bovine (NP_040788); budgerigar (NP_848014); crow (YP_529828); finch (YP_529834); goose (NP_849170); hamster (NP_056730); JC (NP_043512); KI Stockholm 60 (ABN09921); murine (NP_041264); murine pneumotropic (NP_041232); SV40 (NP_043127).

STAg: African green monkey (NP_848009); baboon polyomavirus 1 (YP_406556); BK (YP_717941); bovine (NP_040789); budgerigar (NP_848015); crow (YP_529829); finch (YP_529835); goose (NP_849171); hamster (NP_056732); JC (NP_043513); KI Stockholm 60 (ABN09920); murine (NP_041266); murine pneumotropic (NP_041233); SV40 (NP_043128).

VP1: African green monkey (NP_848007); baboon polyomavirus 1 (YP_406554); BK (YP_717939); bovine (NP_040787); budgerigar (NP_848013); crow (YP_529827); finch (YP_529833); goose (NP_849169); hamster (NP_056733); JC (NP_043511); KI Stockholm 60 (ABN09917); murine (NP_041267); murine pneumotropic (NP_041234); SV40 (NP_043126).

VP2: African green monkey (NP_848005); baboon polyomavirus 1 (YP_406552); BK (YP_717937); bovine (NP_040785); budgerigar (NP_848011); crow (YP_529825); finch (YP_529831); goose (NP_849167); hamster (NP_056734); JC (NP_043509); KI Stockholm 60 (ABN09918); murine (NP_041268); murine pneumotropic (NP_041235); SV40 (NP_043124).

WU complete genome sequences have been deposited under accession numbers EF444549–EF444554. VP2 partial sequences have been deposited under accession numbers EF444555–EF444593.

Acknowledgments

We would like to thank Monique Gaudreault-Keener for technical support and Rakesh Nagarajan and Sunita Koul for assistance with sequence analysis.

Author contributions. MDN, TPS, and DW conceived and designed the experiments. AMG, DMW, IMM, and GW performed the experiments. AMG, MDN, SBL, GAS, TPS, and DW analyzed the data. MDN, DCB, GAS, and TPS contributed reagents/materials/analysis tools. DW wrote the paper.

Funding. This work was supported in part by National Institutes of Health (NIH) grant U54 AI057160 to the Midwest Regional center of Excellence for Biodefense and Emerging Infectious Diseases Research, the Pilot Sequencing Program sponsored by the Center for Genome Sciences at Washington University (DW), and NIH grant K24 DK02886 (DCB). The Brisbane studies were supported by the Royal Children's Hospital Foundation Grant I 922–034, sponsored by the "Woolworths Fresh Futures" Appeal.

Competing interests. AMG, MDN, GW, TPS, and DW are listed on a patent application to the United States Patent Office entitled "Novel Human Polyomavirus."

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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. 5. 2	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況		公表国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)		ProMED 20070501-1414, 2007 May 1. 情報源:Jamaica Observer, 2007 May 1.	ジャマイカ

研究報告の概要

○マラリアージャマイカ(キングストン)新規症例の報告

ジャマイカ保健省によると、2007年4月初めからの1ヶ月間に新規のマラリア症例11例が報告された。2例が4月15日～21日、3例が4月8日～14日、6例が4月1日～7日に報告された。感染者の年齢は10歳～59歳だった。4月22日～27日に報告された2例は、メスのハマダラカが媒介する熱帯熱マラリアで、デンハム・タウンとグリニッジ・タウンで報告され、それぞれの地域で初の報告となった。発症日はそれぞれ4月4日と9日だった。

4月30日、保健省はセントエリザベスで発生した症例は1月以降4例にとどまっていることを示し、他の地域へのマラリア感染拡大を抑制することができたと話した。また、2006年12月に最初の症例が報告されて以降、スーダン、インド、ハイチ、ホンジュラス、ウガンダからの輸入感染症例が7例あったことを指摘した。加えて、4月1日～27日の間に実施された血液検体884の検査陽性率は0.7%～1.8%だったことを説明し、陽性サンプルの数は減少を続けていることを示した。

一方で保健省は、最近の検査でデュアニー川周辺で捕獲されたAnopheles albimanus蚊が、媒介蚊撲滅のために使用されている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過程であると述べた。この検査は米国疾病予防対策センター(CDC)の指導で行われた。

保健省は「引き続き、集中的なサーベイランス、媒介蚊の抑制、市民の教育に力を入れ、マラリア流行を収束させるために組織横断的体制で協力していく。他地域へのマラリア感染拡大を予防するための措置が実施されている」と説明した。

報告企業の意見	今後の対応
ジャマイカの首都キングストンでマラリアが発生しており、4月の1ヶ月間に新規症例11例があったとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の供血を一定期間延期している(1～3年の延期を行うとともに、帰国後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

使用上の注意記載状況・その他参考事項等

白血球除去赤血球「日赤」
照射白血球除去赤血球「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

(17)

Archive Number 20070501.1414
Published Date 01-MAY-2007
Subject PRO/EDR> Malaria - Jamaica (Kingston) (07)

MALARIA - JAMAICA (KINGSTON) (07)
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A ProMED-mail post
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International Society for Infectious Diseases
<http://www.isid.org>

Date: Tue 1 May 2007
Source: Jamaica Observer [edited]
<http://www.jamaicaobserver.com/news/html/20070426T190000-0500_122251_OBS___NEW_CASES_OF_MALARIA_REPORTED.asp>

New cases of malaria reported

A total of 11 new cases of Malaria has been reported since the beginning of April [2007], the Ministry of Health said on 30 Apr 2007.

According to a release from the ministry, 2 cases were reported between 15-21 Apr [2007], 3 the previous week [15-21 Apr 2007] while a total of 6 cases was reported between 1-7 Apr [2007]. The ages of the affected persons range from 10 to 59.

Concerning the 2 new cases reported last week [22-27 Apr 2007], the ministry said they were found in Denham Town and Greenwich Town -- 2 of the areas in which the disease was 1st detected -- and were caused by the _Plasmodium falciparum_ parasite which is transmitted by the female _Anopheles_ mosquitoe. The dates of onset were said to be 4 and 9 Apr [2007] respectively.

Yesterday [30 Apr 2007], the ministry said it had been able to limit the spread of malaria to other parishes, noting that the 4 cases detected in St. Elizabeth since January [2007] remained contained. The health ministry also pointed out that since the 1st case of malaria was reported last December [2006], there have been 7 imported cases originating from Sudan, India, Haiti, Honduras and Uganda.

Additionally, it said the number of positive samples continues to decline, explaining that the positivity rate of blood samples submitted to laboratories over the past weeks range between 0.7 and 1.8 percent from a total of 884 sample tests conducted between 1-21 Apr [2007].

Meanwhile, the ministry reiterated that it was in the process of seeking alternative insecticides to prevent further outbreaks after recent tests confirmed some resistance of the _Anopheles albimanus_ mosquito taken from the Duhaney River to malathion insecticide, which it was hoping to use to eliminate the parasites. The tests were

conducted by consultants from the United States-based Centers for Disease Control and Prevention (CDC).

"The Ministry of Health continues its thrust in the areas of intense active surveillance, vector control, public education and inter-sectoral collaboration in a concerted effort to end this outbreak, while precautionary measures are being taken to prevent the spread of malaria to other parishes," the ministry, however, assured.

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[We assume that some patients have had more than one sample, and the 884 positive samples therefore represents a lower number of patients. We reported on 9 Apr 2007 that 340 people had been infected; and it would be interesting to know the number of cases and not only the number of malaria-positive blood films to know whether the outbreak is under control. □ Mod. EP]

[see also:
Malaria - Jamaica (Kingston) (06) 20070409.1190
Malaria - Jamaica (Kingston) (05) 20070210.0515
Malaria - Jamaica (Kingston) (04) 20070208.0500
Malaria - Jamaica (Kingston) (03) 20070127.0358
Malaria - Jamaica (Kingston) (02) 20070112.0149
Malaria - Jamaica (Kingston): RFI 20070111.0132
2006

Malaria - Jamaica (Kingston) (03) 20061228.3640
Malaria - Jamaica (Kingston) (02): P. falciparum 20061207.3451
Malaria - Jamaica (Kingston): RFI 20061205.3427
Malaria - Bahamas (Exuma Islands) 20060620.1705
2005

Malaria - Haiti, Canada ex Haiti (02): Cotes des Arcadins 20051115.3340
Malaria - Haiti, Canada ex Haiti 20051111.3292
2004

Malaria ex Dominican Republic (02) 20041211.3282
Malaria ex Dominican Republic 20041202.3217
Malaria, imported - Europe ex Dominican Rep. 20041128.3176
2001

Malaria - Italy ex Dominican Republic 20010604.1101
2000

Malaria - Dominican Republic: update (02) 20000310.0326
Malaria - Dominican Republic: update: CORRECTION 20000224.0251
1999

Malaria, imported - Europe ex Dominican Rep. (05) 19991223.2201
1996

Malaria - Haiti 19960502.0846
Haitian, Cuban refugee health: RFI 19960405.0649]
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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. 4. 24	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		ABC Newsletter. 2007 Apr 13.	米国

研究報告の概要

○輸血関連死亡症例数の報告
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不適合以外の溶血性副作用、輸血関連心過負荷、感染症伝播、アナフィラキシーなど)67例(30.6%)、細菌感染20例(9.1%)、ABO不適合による溶血性副作用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。

報告企業の意見	今後の対応
2004年度から2006年度にかけて米国食品医薬品局に報告された輸血副作用による死亡症例数である。	日本赤十字社では、薬事法及び関連法令に従い輸血副作用の情報を収集し、医薬品医療機器総合機構を通じて国に報告している。今後も引き続き輸血副作用に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

別紙3

20

JOURNALISTIC PRODUCT DEVIATIONS: Transfusion Fatalities

An incorrect category label in the table of transfusion recipient fatalities reported to the Food and Drug Administration, published in the March 30 issue, has caused some confusion. This category was erroneously called "Non-ABO Hemolytic Reactions (K, JKa, FYa, etc.)." It should have been titled "Other Reactions: (Non-ABC hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)." Corrected tables follow.

Transfusion Recipient Fatalities Reported to the Food and Drug Administration, FY2004 - 2006

CATEGORIES	FY04	FY05	FY06
TRALI	21 *30.9%*	30 *36.6%*	35 *50.7%*
Other Reactions: (Non-ABO hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)	20 29.4%	25 30.5%	22 31.9%
Bacterial Contamination	6 *8.8%*	9 *11.0%*	5 *7.2%*
ABO Hemolytic Transfusion Reaction	7 *10.3%*	5 *6.1%*	3 *4.3%*
Transfusion not Ruled Out	14 *20.6%*	13 *15.9%*	4 *5.8%*
TOTAL	68	82	69

Total Fatalities

CATEGORIES	FY04 to FY06	Average/Year
TRALI	86 *39.3%*	29
Other Reactions: (Non-ABO hemolytic reactions, TACO, infectious disease transmission, anaphylaxis, etc.)	67 *30.6%*	22
Bacterial Contamination	20 *9.1%*	7
ABO Hemolytic Transfusion Reaction	15 *6.8%*	5
Transfusion not Ruled Out	31 *14.2%*	10
TOTAL	219	73

Data source: Leslie Holness, MD, Office of Blood Research and Review, Food and Drug Administration, Personal Communication, 1/24/07

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. 5. 15	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況	T. Sasahara, Y. Morisawa, A. Yoshimura, S. Hayashi, H. Gomi, Y. Hirai. American Society for Microbiology 107th Annual Meeting; 2007 May 21-25; Toronto.	公表国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)			日本

研究報告の概要

○高度に汚染されたリネン類を原因とした病院内*Bacillus cereus*血流感染アウトブレイク

背景: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、入院患者と外来患者双方の全培養結果のモニタリングが感染症専門医により行われた。2006年8月1日～8月31日までの期間に、患者28名の血液培養から*Bacillus cereus*が検出された。当院での過去2年間の培養結果のベースラインデータに基づき、これらの症例をアウトブレイクとみなした。月間の*Bacillus*菌陽性血液培養検体数および患者数の平均±標準偏差は、それぞれ2.52±2.12、2.28±1.90であった。

方法:*B. cereus*が検出された患者28名の医療記録を調査した。患者のリネン類(タオル、シーツ)、止血帯、皮膚清浄綿、皮膚滅菌剤、看護師が注入用液剤を混合するワゴン台表面、手指消毒剤、血液培養ボトルおよび注入ラインから得られた環境検体の培養を行った。検体は65℃で20分間熱処理後、血液寒天培地に接種した。37℃で48時間培養した後にプレートを調べた。

結果:試験期間中8名の患者に「真の」*B. cereus* 菌血症の可能性を認めた。1名は眼内炎を発症し、2名は死亡した。末梢静脈ライン以外に共通した曝露はなかった。1例の注入ラインからは、大量の*B. cereus*が生育した。また、リネン類から得られた*B. cereus*の平均菌数(CFU/㎠)は、タオルが18,400 、シーツが103であった。他の検体からはほとんど検出されなかった。汚染されたリネン類は、特定の大容量連続槽洗濯機で洗浄されており、これが汚染を拡大したことが判明した。一時的にリネン類のオートクレーブ処理を行い(1回)、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。

結論:当院における*B. cereus* 血流感染アウトブレイクは、高度に汚染されたリネン類と末梢静脈ラインの不適切な取り扱いによって発生したと考えられた。複数の方法によって当該アウトブレイクを収束させた。

報告企業の意見	今後の対応
日本の自治医科大学病院における*Bacillus cereus*の集団院内感染は、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との報告である。輸血後細菌感染症の調査には、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	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

白血球除去赤血球「日赤」
照射白血球除去赤血球「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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Nosocomial Outbreak of *Bacillus cereus* Bloodstream Infection Caused by Highly Contaminated Linens

T. Sasahara, Y. Morisawa, A. Yoshimura, S. Hayashi, H. Gomi, Y. Hirai;
Jichi Medical Univercity, Tochigi, JAPAN.

Presentation Number: L-004
Poster Board Number: 288
Keyword: Bacillus cereus, Contaminated linen, Bloodstream infection

Background: In the Jichi Medical University Hospital, a tertiary care center with 1082 beds in Japan, all the culture results obtained from both inpatients and outpatients have been monitored by infectious disease physicians. *Bacillus cereus* grew from blood cultures obtained from 28 patients between April 1 and August 31, 2006. We considered these cases as an outbreak on the basis of our baseline data on all culture results for the past 2 years; the average +/- standard deviation numbers of *Bacillus* species-positive blood culture specimens and patients per month had been 2.52 +/- 2.12 and 2.28 +/- 1.90, respectively. Methods: We reviewed the charts of 28 patients whose blood cultures grew *B. cereus*. Environmental cultures obtained from linens (towels and bed sheets), tourniquets, stocked skin prep swabs, skin disinfectants, ward table surfaces for nurses' mixing infusion solutions, hand sanitizers, blood culture bottles, and infusion lines from selected patients. Samples were inoculated into blood agars after 65°C heat-treatment for 20 min. Plates were examined after 48-h incubation at 37°C. Results: During the period of investigation, we found that 8 patients had possibly "true" *B. cereus* bacteremia. One patient had endophthalmitis and 2 patients had died. There were no common exposures excluding peripheral venous lines. An infusion line of one case grew a large quantity of *B. cereus*. In addition, the average numbers of CFU/cm^2 of *B. cereus* from linens were 18,400 for towels and 103 for bed sheets, respectively. Few were detected from other environmental specimens. The contaminated linens were handled with a specific large continuation tank washing machine, which was determined to have amplified contamination. By temporarily autoclaving linens (only once), descaling the washing machine, and staff education on peripheral venous line management, there were no additional *B. cereus*-positive blood cultures afterward. Conclusions: In our hospital, *B. cereus* bloodstream infection outbreak occurred probably due to highly contaminated linens and improper handling of peripheral venous lines. We contained the outbreak by a multi-modal approach.

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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. 5. 15	該当なし	

一般的名称	白血球除去人赤血球浮遊液	研究報告の公表状況	Rupp ME, Sholtz LA, Jourdan DR, Marion ND, Tyner LK, Fey PD, Iwen PC, Anderson JR. Clin Infect Dis. 2007 Jun 1;44(11):1408-14. Epub 2007 Apr 16.	公表国
販売名(企業名)	白血球除去赤血球「日赤」(日本赤十字社) 照射白血球除去赤血球「日赤」(日本赤十字社)			米国

研究報告の概要

○無針静注バルブの導入に伴う血流感染のアウトブレイク
背景:針刺し事故のリスクを最小限にするため無針静注カテーテルコネクターバルブが臨床現場に導入されている。しかし、これらバルブに関連する感染管理のリスクは過小に評価されている。2005年3月、ある病院の複数の病室において、特定の無針バルブが導入されていた期間中に血流感染の急激な増加が認められた。
方法:標準的方法を用い、病院全体の1次血流感染のサーベイランスを実施した。血液培養の細菌汚染率をモニターした。静注カテーテルコネクターバルブから採取したサンプルを用いて培養を実施した。
結果:ベースラインと比較した当該コネクターバルブ導入期間中の血流感染の相対リスクは2.79 (95%信頼区間、2.27-3.43)であった。重症集中治療室では一次血流感染の割合は、同バルブ導入によってカテーテル留置1000日当り3.87感染から10.64感染に増加し(P<0.001)、バルブ使用中止後の6ヶ月間では5.59感染(P=0.02)に減少した。同様に、入院看護室では、同バルブ導入によってカテーテル留置1000日当り3.47感染から7.3感染に増加したが(P=0.02)、使用中止後の6ヶ月間は2.88感染(P=0.57)に減少した。チーム治療室でも同様の事象が発生した。血液培養の細菌汚染率は、試験期間中それほど変わらなかった。細菌検体検査に供した37バルブのうち、24.3%に細菌が生育し、そのほとんどはコアグラーゼ陰性ブドウ球菌であった。
結論:一次血流感染と無針コネクターバルブとの有意な関連性が認められた。無針コネクターバルブの検討には、市場導入前に前向きランダム化試験で感染リスクを十分評価することを含めるべきである。

報告企業の意見	今後の対応
米国ネブラスカ州の病院で複数の病室において、特定の無針静注カテーテルコネクターバルブが導入されていた期間に血流感染の急激な増加が見られ、一次血流感染と無針コネクターバルブの有意な関連性が認められたとの報告である。輸血後細菌感染症の調査には、院内感染など輸血以外の伝播ルートについて考慮する必要がある。	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

白血球除去赤血球「日赤」
照射白血球除去赤血球「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

MAJOR ARTICLE

Outbreak of Bloodstream Infection Temporally Associated with the Use of an Intravascular Needleless Valve

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Background. Needleless intravascular catheter connector valves have been introduced into clinical practice to minimize the risk of needlestick injury. However, infection-control risks associated with these valves may be underappreciated. In March 2005, a dramatic increase in bloodstream infections was noted in multiple patient care units of a hospital in temporal association with the introduction of a needleless valve into use.

Methods. Surveillance for primary bloodstream infection was conducted using standard methods throughout the hospital. Blood culture contamination rates were monitored. Cultures were performed using samples obtained from intravascular catheter connector valves.

Results. The relative risk of bloodstream infection for the time period in which the suspect connector valve was in use, compared with baseline, was 2.79 (95% confidence interval, 2.27–3.43). In critical care units, the rate of primary bloodstream infection increased with the introduction of the valve from 3.87 infections per 1000 catheter-days to 10.64 infections per 1000 catheter-days ($P < .001$), and it decreased to 5.59 infections per 1000 catheter-days ($P = .02$) in the 6 months following removal of the device from use. Similarly, in inpatient nursing units, the rate of bloodstream infection increased from 3.47 infections per 1000 catheter-days to 7.3 infections per 1000 catheter-days ($P = .02$) following introduction of the device, and it decreased to 2.88 infections per 1000 catheter-days ($P = .57$) following removal of the device from use. Similar events occurred in the cooperative care units. The rate of blood culture contamination did not substantially change over the course of the study. Of 37 valves that were subjected to microbiological sample testing, 24.3% yielded microbes, predominantly coagulase-negative staphylococci.

Conclusion. A significant association between primary bloodstream infection and a needleless connector valve was observed. Evaluation of needleless connector valves should include a thorough assessment of infection risks in prospective randomized trials prior to their introduction to the market.

Needleless intravascular access systems are mandated to reduce the risk of needlestick injuries in health care workers [1, 2]. There are 3 basic design types of needleless access systems: split-septum connectors; luer-activated valves; and positive-displacement, luer-activated valves. There are numerous commercially available products within each basic design type. Many of these needleless access systems are introduced into clinical use without thorough evaluation of associated infection control risks. Recently, concerns have been expressed regarding increased rates of bloodstream infection associated with the use of newer needleless mechanical valve systems [3–5].

Intravascular catheter–related bacteremia is a substantial clinical problem that results in an attributable mortality of ~3% and an attributable cost-per-incident of ~$25,000 among the estimated 250,000 patients annually who experience this complication in the United States [6–9]. Therefore, if any portion of the intravascular access system increases the risk of bloodstream infection, it must be thoroughly evaluated, and clinicians should be appropriately alerted.

A dramatic increase in the rate of primary blood-

Received 12 January 2007; accepted 21 February 2007; electronically published 16 April 2007.

Presented in part: 16th Annual Meeting of the Society for Healthcare Epidemiology of America, Chicago, Illinois, March 2006 (abstract 227).

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Clinical Infectious Diseases 2007; 44:1408–14

1058-4838/2007/4411-0002$15.00
DOI: 10.1086/517538

stream infection in multiple inpatient units was observed in our institution in temporal association with the introduction of a positive-displacement, luer-activated, needleless connector valve. Similarly, upon removal of the putative offending device, the rate of bloodstream infection decreased. Herein, we report these findings and other observations supporting the causative role of the intravascular needleless connector valve in the outbreak.

METHODS

Location and surveillance methods. The Nebraska Medical Center (Omaha) is a 689-bed academic medical center. Continuous active surveillance for bloodstream infections in critical care units and cooperative care units (step-down care facilities for bone marrow and solid-organ transplant recipients in which a patient and a care partner [a spouse or family member] are housed together in a home-like environment) has been conducted for years using Centers for Disease Control and Prevention methods and definitions [10]. Surveillance for device-associated bloodstream infections was implemented in other nursing units in November 2004. Initially, surveillance was conducted every third month; in May 2005, continuous surveillance was instituted. Bloodstream infection rates were monitored in 3 types of patient care areas: critical care and transplantation units (8 patient care units consisting of 132 beds), inpatient nursing units (9 patient care units consisting of 312 beds), and transplantation cooperative care units (2 inpatient care units consisting of 22 beds). A primary bloodstream infection was defined as occurring when ≥1 blood culture of samples obtained from a patient yielded a pathogen that was not present because of an infection at another site. Common skin contaminants (e.g., diptheroids or coagulase-negative staphylococci) were disregarded, unless they were recovered from ≥2 blood samples that were obtained separately or from a patient who had a central venous catheter and for whom the physician instituted appropriate antimicrobial therapy. Infections manifesting within 48 h after admission to the hospital were regarded as non–hospital acquired. Primary bloodstream infection rates were expressed in infections per 1000 central venous catheter–days. In the cooperative care units, accurate intravascular catheter census data were not reliably available, and the infection rates were expressed in infections per 1000 patient-days.

Devices and timing of clinical use. Prior to February of 2005, a split-septum intravascular access connector valve (Interlink IV Access System; Baxter) was in use in our hospital. During the last week of February 2005, a luer-activated, positive-displacement, intravascular access valve (SmartSite Plus; Alaris Medical Systems) was introduced into clinical practice throughout the hospital. Education on the proper use of the intravascular access valve was conducted on all units by nurse educators and manufacturer's representatives. No changes were instituted in catheter insertion or care protocol during the observation periods. The intravenous administration set and connector valves were changed every 7 days, or more frequently if the connector valve or tubing appeared to be damaged, showed signs of leakage, or was visibly contaminated with blood [9]. Administration sets were changed more frequently when blood products, lipids, or parenteral nutrition formulations were infused [9]. Intravenous access ports were cleaned with a swab containing 70% isopropyl alcohol before accessing the port. Following an observed increase in the rate of bloodstream infections, efforts to replace the positive-displacement intravascular access valve were initiated in late June 2005, and the previously used split-septum valve was returned to use throughout the hospital by 1 September 2005. Although there was widespread institutional recognition of the outbreak and increased vigilance with regard to compliance with vascular access insertion and care protocol, a formal, system-wide reeducation effort was not conducted during the period when the positive-displacement intravascular access valve was in use.

Blood culture contamination. The clinical microbiology laboratory routinely monitored the rate of blood culture contamination using a laboratory definition of contamination. Blood cultures were considered to be most likely contaminated when single blood cultures (1 of 1 blood culture) yielded *Bacillus* species, aerobic and anaerobic diptheroids (including *Corynebacterium* species and *Propionibacterium acnes*), or *Micrococcus* species. If a single blood culture among multiple blood cultures performed using samples obtained from the same patient yielded coagulase-negative staphylococci, it was regarded as being a likely contaminated specimen.

Catheter connector valve cultures. Intravascular access valves were removed from the central venous catheters of 12 adult critical care unit patients and replaced with new valves. The used valves were transported to the laboratory in individual sterile containers. The diaphragms of the used valves were disinfected with 70% isopropyl alcohol wipes (Kendall), and 1 mL of trypticase soy broth (Bacto; Difco Laboratories) was injected through the valves and collected in sterile 5-mL plastic test tubes. A 0.1 mL aliquot of the broth was inoculated onto trypticase soy agar plates (Difco Laboratories), and the plates and remaining broth were incubated at 37°C for 48 h. Standard microbiological testing techniques were used to identify recovered microbes.

Statistical analysis. Primary bloodstream infection rates were modeled over the 3 time periods (baseline [before March 2005], outbreak [March 2005–August 2005], and follow-up [September 2005–February 2006]) for the 3 types of patient care units (critical care and transplantation units [8 areas], inpatient nursing units [9 areas], and transplantation cooperative care units [2 areas]). The number of bloodstream infec-

tions that were observed was modeled using Poisson regression, with the rates assumed to be constant for each of the 6 patient-care-unit–time-period combinations. SAS GENMOD software, version 9.1.3 (SAS) was used for modeling. Comparisons of patient care units and time periods were expressed as relative risks.

RESULTS

As is illustrated in figure 1, the rate of bloodstream infection increased dramatically in all types of patient care areas in conjunction with the introduction of the positive-displacement connector valve. In the 8 critical care and transplantation units, the baseline bloodstream infection rate, which was calculated on the basis of 38,250 central venous catheter–days over a 26-month period, was 3.87 infections per 1000 central venous catheter–days. During the 6-month period when the positive-displacement connector valve was in clinical use in our facility (accounting for 10,340 days of central venous catheter use), the rate of bloodstream infection increased 2.82-fold (95% CI, 2.21-fold to 3.61-fold) to 10.64 infections per 1000 central venous catheter–days ($P < .001$). In the 6 months following the discontinuation of use and the removal of the positive-displacement connector valves, the bloodstream infection rate decreased to 5.59 infections per 1000 central venous catheter–days ($P = .02$, compared with baseline). In the 9 inpatient nursing units, the baseline rate of catheter-associated bloodstream infection, which was calculated on the basis of 2 one-month-long observation periods (November 2004 and February 2005) that involved 3745 central venous catheter–days, was 3.47 infections per 1000 central venous catheter–days. During the outbreak period, the rate of bloodstream infection increased 2.1-fold (95% CI, 1.15-fold to 3.86-fold) to 7.3 infections per 1000 central venous catheter–days ($P = .02$). During the postintervention period, the rate of bloodstream infection decreased to 2.88 infections per 1000 central venous catheter–days during 11,475 days of central venous catheter use, which is a similar rate to that observed at baseline ($P = .57$). Finally, in the 2 cooperative care transplantation populations, the baseline rate of bloodstream infection of 5.31 infections per 1000 patient-days that was demonstrated during 7535 patient-days of observation over 26 months of time increased 2.86-fold (95% CI, 1.69-fold to 4.85-fold) to 15.18 infections per 1000 patient-days during 1383 days of patient observation ($P < .001$). This rate decreased to 3.82 infections per 1000 patient-days over 1047 patient-days of observation in the postintervention period, which is a similar rate to that observed at baseline ($P = .53$). There was no statistical evidence that the increased risk differed across the 3 patient care units. The estimated relative risk of bloodstream infection for the 6-month period in which the positive-displacement connector valve was used in our facility,

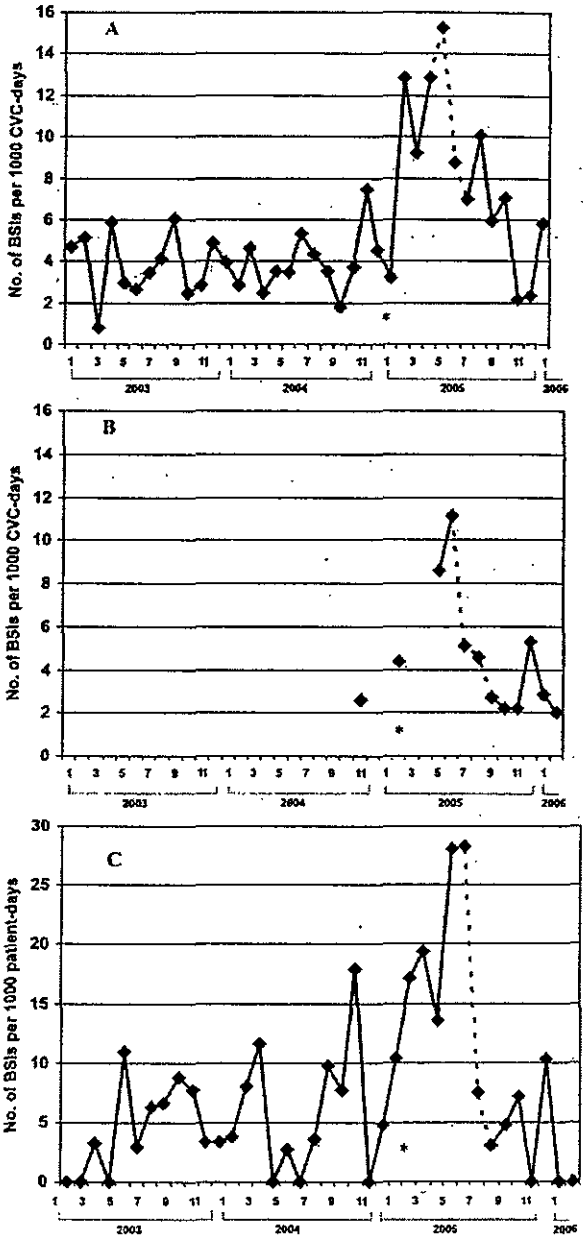

Figure 1. Rate of bloodstream infection versus time, January 2003 to February 2006. Numbers 1–12 in the x-axis refer to consecutive months (from January to December) of the year indicated. Bloodstream infections in critical care units (*A*), in inpatient nursing units (*B*), and in cooperative care units (*C*). Infections are expressed as bloodstream infections (BSI) per 1000 central venous catheter (CVC)–days in panels *A* and *B* and as bloodstream infections per 1000 patient-days in panel *C*. *Asterisk*, when the positive-pressure displacement valve was introduced; *dotted trend line*, the transition period as the valves were removed from clinical use. In panel *B*, the first 2 data points indicate separate observation months in November 2004 and February 2005; continuous surveillance was instituted in May 2005. The connector valve was introduced in late February 2005 and was completely removed from clinical use by September 2005.

compared with the baseline period, was 2.79 (95% CI, 2.27–3.43).

Table 1 documents the microbiological characteristics of the bloodstream infections that were observed during the overall investigation period. In the 26-month preoutbreak period, 201 bloodstream infections were defined, of which 24 (11.9%) were polymicrobic. Sixty-four percent of the infections were due to gram-positive cocci, and 33% were due to coagulase-negative staphylococci. Microbiological characteristics during the outbreak period were quite similar to those in the preoutbreak period. A total of 189 bloodstream infections were observed, of which 16 (8.5%) were polymicrobic. Sixty-four percent of the infections were due to gram-positive cocci and 34% were due to coagulase-negative staphylococci. In the postoutbreak period, 98 bloodstream infections were observed, of which 5 (5.1%) were polymicrobic. The proportion of bloodstream infections due to gram-positive cocci increased to 76%, and coagulase-negative staphylococci caused 45% of the infections. There was no substantial difference in the proportion of polymicrobic bloodstream infections during the observation periods ($P = .15$, by Fisher's exact test). The proportion of infections due to *Candida* species remained fairly constant throughout the periods of observation, at 6%, 6%, and 7% during the preoutbreak, outbreak, and postoutbreak periods, respectively.

The rate of blood culture contamination remained relatively constant over the course of the outbreak. In the 14 months prior to the outbreak, the rate of blood culture contamination was 3.00% (816 contaminated cultures of 27,172 blood samples obtained). During the 6-month outbreak period, the contamination rate was 3.02% (415 contaminated cultures of 13,742 blood samples obtained); during the 6-month follow-up period, the contamination rate was 3.13% (407 contaminated cultures of 12,994 blood samples obtained). The number of blood samples obtained per month increased by 18%, from 1941 samples per month during the preoutbreak period to 2290 samples per month during the outbreak period. In the 6 months following the outbreak, the number of samples obtained per month decreased modestly to 2166 samples per month.

Samples obtained from 9 (24.3%) of 37 positive-displacement intravascular catheter access valves in 7 (58.3%) of the 12 patients yielded positive results when cultured; all valves from multilumen catheters were sampled. The catheters had been inserted an average of 8 days before sampling (median, 5 days; range, 1–27 days). As previously noted, connector valves were routinely changed at 7-day intervals. All positive sample cultures yielded typical skin flora (e.g., coagulase-negative staphylococci or diptheroids) ranging in quantity from 10 to 1500 colony forming units/mL of broth flush solution. No gram-negative bacilli or yeast were recovered from the connector valves. In 1 instance, it was noted that the broth was bloody after being flushed through the connector valve.

DISCUSSION

Needleless vascular connectors have been widely introduced throughout the health care system in response to mandates for improvement in health care worker safety and avoidance of bloodborne pathogen exposure [1, 2]. Split-septum devices were the first needleless devices to be introduced to the market,

Table 1. Microbiological characteristics of central venous catheter–associated bloodstream infections (BSIs).

Pathogen	Percentage of total BSIs caused by specific pathogen		
	Preoutbreak period (n = 201)	Outbreak period (n = 189)	Postoutbreak period (n = 98)
Gram-positive cocci	64	64	76
Coagulase-negative staphylococci	33	34	45
Enterococcus species	19	19	19
Staphylococcus aureus	7	7	8
Other gram-positive cocci	5	4	4
Gram-negative bacilli	25	27	16
Enterobacter species	6	3	1
Klebsiella species	7	6	4
Escherichia coli	2	5	3
Pseudomonas species	2	8	4
Serratia species	2	2	1
Other gram-negative bacilli	6	3	3
Candida species	6	7	6
Other	5	2	2

and consist of a prepierced diaphragm that is accessed via a blunt cannula. Luer-activated devices control an antireflux valve and are compatible with standard twist-lock connector tubing or syringes. Most recently, to minimize catheter occlusion, positive-displacement devices have been introduced that expel a small volume of flush solution back into the catheter when the device is disconnected. Although needleless connectors have been shown to reduce the risk of needlestick injury [11, 12], the benefit of early-generation needleless connectors was questioned after reports of increased rates of primary bloodstream infections associated with their use [13–16]. Investigation of these outbreaks revealed that the risk of infection was increased when connectors were changed less frequently than is recommended, when specific infusates (such as total parenteral nutrition or lipids) were administered, and when other independent factors were considered (such as patient race and education, multilumen catheter use, or recent hematopoetic stem cell transplantation) [13–17]. In 2002, the Centers for Disease Control and Prevention reported that needleless connectors do not substantially affect the incidence of bloodstream infection when they are used according to manufacturer's recommendations [9]. More recently, increasingly sophisticated mechanical valve connectors have been introduced into clinical practice. Limited data are available regarding the risk of bloodstream infection that is associated with these devices. In studies comparing luer-activated valves with simple caps, rates of contamination and bloodstream infection were reduced with the use of the mechanical valves [18–20]. In 2004, Hall et al. [3] first reported infection-related concerns regarding the newer devices; reports from other institutions followed [4–5, 21, 22]. The device implicated in the outbreak described by Maragakis et al. [5] is the same brand of device temporally associated with the increase in rate of bloodstream infection described in this report. In addition, Shilling et al. [23] noted higher catheter occlusion rates associated with the use of a positive displacement needleless valve, compared with a simpler mechanical valve, as well as a trend toward higher infection rates when saline was used to flush the device.

The present report adds to the increasing attribution of unintended consequences to the introduction of various mechanical needleless catheter connectors into use. Several features of our study merit emphasis. There was a striking temporal relationship between introduction of the connector valve and a ~2.7-fold increase in primary bloodstream infection. Similarly, when the connector valve was removed from clinical use in our facility, the rate of bloodstream infection decreased toward baseline. The increase in bloodstream infection was quickly detected and was observed in all areas and in all patient groups where the device was used. The rapid detection of this outbreak emphasizes the value of well-functioning systems of surveillance for health care–associated infection. The widespread nature of the outbreak indicated a generalized problem, not one associated with a small group of health care workers or a limited point-source outbreak. As illustrated in table 1, the microbiologic etiology of the bloodstream infections was relatively constant and was most consistent with inoculation of the intravascular catheter system with skin flora. The variety of microbial species that were isolated and the lack of reports of similar outbreaks in the region argues against a generalized contamination of infusate as the causative factor. Likewise, no change in the protocol of intravascular catheter insertion or care occurred that could explain a generalized outbreak. Finally, additional support for the causative role of the connector valve came from the results of microbiological testing of samples obtained from the connector valves in clinical use. Microbes were recovered from 24.3% of the sampled connector valves. In comparison, 2 clinical studies examining the risk of microbial colonization of connector valves [18, 20] reported rates of colonization of 4.3% of patients and 6.6% of devices, whereas Danzig et al. [15] related a connector device colonization rate of 21.7% associated with a bloodstream infection outbreak.

Limitations of this study must also be emphasized. First, this was not a prospective, randomized trial. These data are retrospective, observational, and uncontrolled. In addition, our report details experience at a single institution. Although the connector valve was introduced into clinical use in conjunction with an extensive educational program, it is not known whether further education regarding catheter insertion and care would have ameliorated the outbreak. Although earlier studies [13, 14, 16] have indicated that lapses in intravascular catheter care could explain increased infection rates associated with the introduction of new connector valves, other investigators have found repeated educational efforts regarding proper use of the devices to be unrewarding [4].

Although speculative, we believe that the design of the connector valve introduced in our hospital in March 2005 may have promoted microbial contamination and colonization. Upon close inspection of the valve (figure 2), one can observe a shallow depression and rim between the diaphragm and the plastic housing. It is possible that microbes and debris could collect in this area, which would be relatively resistant to cleansing or disinfection. The internal mechanism of the valve contains moving parts, which introduces irregularities in the fluid flow and may promote areas of stagnation and create potential reservoirs for microbial growth. Also, the plastic housing is opaque, which prohibits visual inspection of the connector valve. Therefore, it is possible that blood or infusion products could collect within the valve and, because of its opaque nature, go unnoticed by health care workers. Last, because of stiffness or "memory" of plastic intravenous tubing, if the luer connection mechanism is not fully engaged, the tubing can untwist, resulting in disconnection and possible contamination.

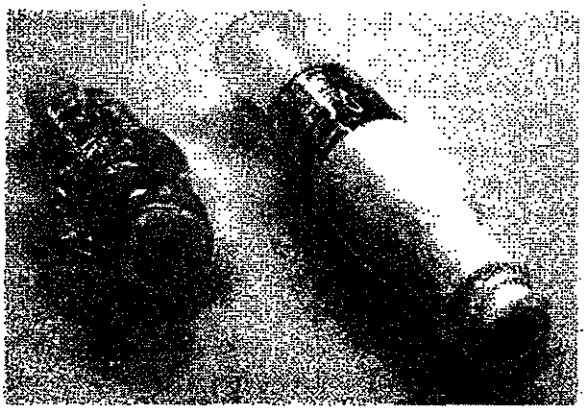

Figure 2. The 2 vascular catheter connector valves described in this study. The valve on the left is the split-septum valve used before and after the outbreak of primary bloodstream infection. The valve on the right is the luer-activated, positive-displacement connector valve that was temporally associated with an increase in the rate of bloodstream infection.

This study also demonstrated the complexities of health care system supply lines. A multitude of persons and viewpoints are involved in decisions regarding supply purchases; potential infection control concerns are only one, albeit a very important, consideration. In heavily bureaucratized and outsourced hospital supply systems, decisions regarding device distribution are not easily communicated throughout. In addition, end users of supplies may have personal stockpiles of supplies that are not officially sanctioned. We encountered obstacles at various levels of the supply chain in attempting to remove the connector valve from clinical use. Although efforts to remove the new connector valve and replace it with the original valve were initiated in June, we continued to find the putative offending device in sporadic clinical use throughout the summer. This may explain why bloodstream infection rates did not decrease as steeply when the device was removed as they had increased when the device was introduced. Only through repeated, thorough searches of supply rooms, bedside cabinets, and nursing units and repeated communication along the supply line did we finally achieve a complete exchange of devices by September.

In conclusion, a strong temporal relationship was observed between the introduction of a positive-displacement intravascular catheter connector valve and an increase in the rate of primary bloodstream infection that resolved when the connector valve was removed from clinical use. Although causation can not be concluded from these data, clinicians and persons responsible for institutional medical device purchase and use should be aware of the association between this particular connector valve and an increased risk for bloodstream infection. Because of significant morbidity, mortality, and economic cost associated with bloodstream infection, any change in the design of intravascular catheter devices or the procedures used in their insertion or care should be thoroughly investigated in adequately sized and well-designed studies to ascertain unanticipated infectious complications before they are approved and introduced to the market. Finally, the value of surveillance systems for the detection of health care–associated adverse events and well-functioning infection-control programs is once again demonstrated.

Acknowledgments

We gratefully acknowledge the diligent efforts of Allen Gould and Jacqueline Parmenter in coordinating the introduction and removal of devices throughout the hospital supply system.

Potential conflicts of interest. M.E.R. has received recent research funding from Cubist Pharmaceuticals, NABI Pharmaceuticals, Becton Dickinson, and 3M Health Care; is a member of the advisory boards of Cubist Pharmaceuticals and Wyeth Laboratories; and is a member of the speakers' bureaus of Cubist Pharmaceuticals and Becton Dickinson. All other authors: no conflicts.

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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. 4. 24	該当なし	

一般的名称	人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	赤血球M・A・P「日赤」(日本赤十字社) 照射赤血球M・A・P「日赤」(日本赤十字社) 赤血球濃厚液-LR「日赤」(日本赤十字社) 照射赤血球濃厚液-LR「日赤」(日本赤十字社)		asahi.com. 2007 Apr 18.	日本

研究報告の概要

○関東ではしかが流行　10代前半や大人に多いのが特徴
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。
3月26日からの1週間に、全国3千の小児科から報告された患者数は26人(昨年同期9人)で、うち22人が東京都と埼玉、千葉、神奈川各県だった。同期間に全国約500の病院を受診した成人の患者数は11人(同0人)で、うち8人が東京。同センターは「この地域のはしかの流行はさらに進行している可能性が高い」としている。
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。
同センターの多屋馨子室長は「例年これからが流行のピークになる。ワクチンを受けていない人は早急に近くの医療機関に問い合わせて受けてほしい」と話している。

報告企業の意見	今後の対応
関東地方ではしか(麻疹)が流行し、10代や大人の患者が多いとの報告である。	日本赤十字社は、輸血感染症対策として、問診ではしか(麻疹)の既往があった場合治癒後3週間献血不可としている。今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

赤血球M・A・P「日赤」
照射赤血球M・A・P「日赤」
赤血球濃厚液-LR「日赤」
照射赤血球濃厚液-LR「日赤」

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

関東ではしかが流行　10代前半や大人に多いのが特徴

2007年04月18日15時18分

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この記事の関連情報

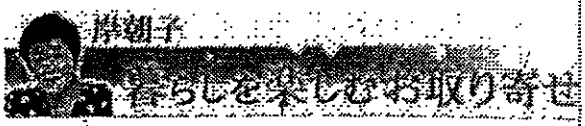
暮らしのページ
医療・病気ニュース

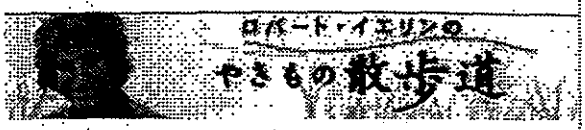
暮らし

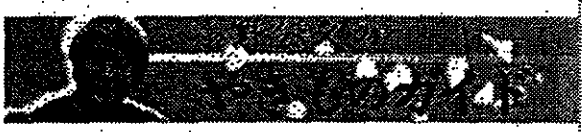
アユ漁解禁　長良川と根尾川の一部で (11:40)
御在所岳でアカヤシオ見ごろ　三重 (07:48)
人気の200系、開業時の塗装で運行　東北・上越新幹線 (06:23)
中高年、携帯電話で若者と親交？「装飾メール」利用増 (20:47)
あぐら姿でお点前いかが　裏千家が考案 (15:57)
死産した母犬が、トラの赤ちゃんに授乳　香川の動物園 (08:03)
大阪・御堂筋、景観条例か超高層で活力か　完成70年 (03:17)
12人が年金を「もらい損ね」　社保庁の納付記録が欠落 (23:39)
温水洗浄便座の発火・発煙事故が、23年間に105件 (21:29)
バストだけでなく、投票率もアップするブラジャー？ (19:30)

暮らし記事一覧

大学院選び：大学全入時代に修士は必要ですか







MY TOWN マイタウン 地域情報

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ポッドキャスト	中之島プロジェクト始動

このページのトップに戻る

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(2007/6/1～2007/8/31)

血対ID	受理日	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正使
70059	2007/06/15	日本赤十字社	人全血液	人全血液	人血液	日本	有効成分	有	無	有
70060	2007/06/15	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液	人血液	日本	有効成分	有	有	有
70061	2007/06/15	日本赤十字社	抗HBs人免疫グロブリン	抗HBs人免疫グロブリン	人血液	日本	有効成分	有	無	無
70062	2007/06/18	化学及血清療法研究所	乾燥スルホ化人免疫グロブリン	スルホ化人免疫グロブリンG	ヒト血液	米国、日本	有効成分	有	無	無
70063	2007/06/18	化学及血清療法研究所	乾燥濃縮人アンチトロンビンⅢ	アンチトロンビンⅢ	ヒト血液	日本	有効成分	有	有	無
70064	2007/06/22	ベネシス	ポリエチレングリコール処理 抗破傷風人免疫グロブリン 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
70065	2007/06/29	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	有	無	無
70066	2007/06/29	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	有	無	無
70067	2007/07/10	富士フイルムRIファーマ	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)	ヒト血液	米国	有効成分	有	無	無
70068	2007/07/18	化学及血清療法研究所	乾燥濃縮人血液凝固第Ⅷ因子	血液凝固第Ⅷ因子	ヒト血液	日本	有効成分	有	無	無
70069	2007/07/18	ベネシス	人ハプトグロビン	人ハプトグロビン	人血液	非献血:米国 献血:日本	有効成分	有	無	無
70070	2007/07/24	日本製薬	乾燥人血液凝固第Ⅸ因子複合体	血液凝固第Ⅸ因子複合体	人血液	日本	有効成分	有	無	無
70071	2007/07/24	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ新生仔血清	ウシ血液	ニュージーランド	製造工程	無	無	無
70072	2007/07/24	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	エプタコグ アルファ(活性型)(遺伝子組換え)	該当しない	有効成分	無	無	無
70073	2007/07/24	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ブタ膵臓由来トリプシン	ブタ膵臓(抽出物)	不明	製造工程	無	無	無
70074	2007/07/24	ノボノルディスクファーマ	エプタコグ アルファ(活性型)(遺伝子組換え)	ウシ胎仔血清	ウシ血液	ニュージーランド、オーストラリア、米国及びカナダ	製造工程	無	無	無
70075	2007/07/26	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	有	無
70076	2007/07/26	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

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
70077	2007/07/26	CSLベーリング	乾燥濃縮人アンチトロンビンⅢ	乾燥濃縮人アンチトロンビンⅢ	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	有	有
70078	2007/07/27	日本赤十字社	白血球除去人赤血球浮遊液	白血球除去人赤血球浮遊液	人血液	日本	有効成分	有	有	有
70079	2007/07/27	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液	人血液	日本	有効成分	有	有	有
70080	2007/07/27	日本赤十字社	人全血液	人全血液	人血液	日本	有効成分	有	無	有
70081	2007/07/27	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液	人血液	日本	有効成分	有	有	有
70082	2007/07/27	日本メジフィジックス	放射性医薬品基準ガラクトシル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	ガラクトシル人血清アルブミンジエチレントリアミン五酢酸テクネチウム(99mTc)	生物学的製剤基準人血清アルブミン	日本	有効成分	有	無	無
70083	2007/07/31	バクスター	加熱人血漿たん白	人血清アルブミン	人血漿	米国	有効成分	有	無	
70084	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ルリオクトコグ　アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	有	有	無
70085	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	人血清アルブミン	人血漿	米国	添加物	有	有	無
70086	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ膵臓	米国	製造工程	無	有	無
70087	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造	ウシ血液	オーストラリア	製造工程	無	有	無
70088	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	アプロチニン	ウシ肺	ニュージーランド	製造工程	無	有	無
70089	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ウシ血清アルブミン	ウシ血液	米国	製造工程	無	有	無
70090	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	培養補助剤（抗第Ⅷ因子モノクローナル抗体製造用-	ウシ血液	米国	製造工程	無	有	無
70091	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	培養補助剤（抗第Ⅷ因子モノクローナル抗体製造用-	ウシ肝臓	米国又はカナダ	製造工程	無	有	無
70092	2007/08/16	富士フイルムRIファーマ	ヨウ化人血清アルブミン(131I)	ヨウ化人血清アルブミン(131I)	ヒト血液	日本	有効成分	有	無	無
70093	2007/08/23	化学及血清療法研究所	乾燥抗破傷風人免疫グロブリン	抗破傷風人免疫グロブリン	ヒト血液	米国	有効成分	有	無	無
70094	2007/08/23	化学及血清療法研究所	乾燥人血液凝固第Ⅸ因子複合体 乾燥濃縮人血液凝固第Ⅸ因子	血液凝固第Ⅸ因子	ヒト血液	日本	有効成分	有	無	無
70095	2007/08/23	化学及血清療法研究所	乾燥人血液凝固第Ⅸ因子複合体 乾燥濃縮人血液凝固第Ⅸ因子 乾燥濃縮人アンチトロンビンⅢ	ヘパリンナトリウム	ブタ小腸粘膜	中国、フランス、米国、カナダ	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
70096	2007/08/24	日本製薬	①加熱人血漿たん白 ②人血清アルブミン(5%) ③人血清アルブミン(20%) ④人血清アルブミン(25%) ⑤乾燥ポリエチレングリコール処理人免疫グロブリン ⑥トロンビン ⑦乾燥濃縮人アンチトロンビンIII ⑧人免疫グロブリン ⑨乾燥人血液凝固第IX因子複合体	ヘパリン	ブタ腸粘膜	ブラジル	①～⑧製造工程 ⑨添加物・製造工程	無	無	無
70097	2007/08/24	ベネシス	人血清アルブミン 乾燥濃縮人アンチトロンビンIII 人ハプトグロビン 乾燥濃縮人血液凝固第VIII因子	ヘパリン	ブタ小腸粘膜	中国	製造工程	無	無	無
70098	2007/08/24	CSLベーリング	人血清アルブミン 人免疫グロブリンG 破傷風抗毒素 フィブリノゲン加第XIII因子 ペプシン処理人免疫グロブリンG 乾燥濃縮人アンチトロンビンIII	ヘパリンナトリウム	ブタ腸粘膜	中国	製造工程	無	無	無
70099	2007/08/24	CSLベーリング	人C1-インアクチベーター	人C1-インアクチベーター	ヒト血液	米国、ドイツ、オーストリア	有効成分	有	無	有
70100	2007/08/31	バクスター	ルリオクトコグ アルファ(遺伝子組換え)	ルリオクトコグ アルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	無	無

別紙様式第4

感染症発生症例一覧

番号	感染症の種類		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
	器官別大分類	基本語								
1	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
2	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
3	感染症および寄生虫症	細菌感染	日本	男	46	2007/3/28	①回復	症例報告	当該製品	2007/4/13 提出、識別番号 1-07000006 未完了報告
4	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
5	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 (4番と同一症例)
6	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
7	感染症および寄生虫症	B型肝炎	日本	女	70	2007/3/13	③未回復	症例報告	当該製品	2007/4/2 提出、識別番号 1-07000001 未完了報告
8	感染症および寄生虫症	B型肝炎	日本	女	70	2007/3/13	③未回復	症例報告	当該製品	2007/4/17提出、識別番号1-07000001 未完了報告 (8番と同一症例)
9	感染症および寄生虫症	B型肝炎	日本	女	70	2007/3/13	③未回復	症例報告	当該製品	2007/4/27提出、識別番号1-07000001 未完了報告 (8番、9番と同一症例)
10	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
11	感染症および寄生虫症	細菌感染	日本	男	1	2007/3/4	③未回復	症例報告	当該製品	2007/3/19 提出、識別番号 1-06000258 未完了報告
12	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
13	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
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	B型肝炎	日本	女	79	2007/2/13	⑥不明	症例報告	当該製品	2007/3/20 提出、識別番号 1-06000260 未完了報告
16	感染症および寄生虫症	B型肝炎	日本	女	79	2007/2/13	③未回復	症例報告	当該製品	2007/4/12提出、識別番号1-06000260 未完了報告 (15番と同一症例)
17	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
18	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
19	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	1	2007/2/6	③未回復	症例報告	当該製品	2007/3/2 提出、識別番号 1-06000233 未完了報告
20	感染症および寄生虫症	C型肝炎	日本	女	50	2007/2/6	③未回復	症例報告	当該製品	2007/3/19 提出、識別番号 1-06000259 未完了報告
21	感染症および寄生虫症	C型肝炎	日本	女	70	2007/2/5	③未回復	症例報告	当該製品	2007/2/22 提出、識別番号 1-06000227 未完了報告
22	感染症および寄生虫症	B型肝炎	日本	女	55	2007/2/1	③未回復	症例報告	当該製品	2007/2/21 提出、識別番号 1-06000224 未完了報告
23	感染症および寄生虫症	B型肝炎	日本	男	17	2007/2/1	③未回復	症例報告	当該製品	2007/2/21 提出、識別番号 1-06000225 未完了報告
24	感染症および寄生虫症	細菌感染	日本	男	74	2007/1/31	①回復	症例報告	当該製品	2007/2/15 提出、識別番号 1-06000219 未完了報告
25	感染症および寄生虫症	ブドウ球菌感染	日本	女	7	2007/1/25	②軽快	症例報告	当該製品	2007/2/22 提出、識別番号 1-06000228 未完了報告
26	感染症および寄生虫症	エプスタイン・バーウイルス感染	日本	女	1	2007/1/24	③未回復	症例報告	当該製品	2007/3/2 提出、識別番号 1-06000234 未完了報告
27	感染症および寄生虫症	C型肝炎	日本	男	69	2007/1/22	③未回復	症例報告	当該製品	2007/2/15 提出、識別番号 1-06000218 未完了報告
28	感染症および寄生虫症	B型肝炎	日本	男	67	2007/1/19	③未回復	症例報告	当該製品	2007/2/6 提出、識別番号 1-06000215 未完了報告
29	感染症および寄生虫症	細菌感染	日本	男	85	2007/1/12	①回復	症例報告	当該製品	2007/4/13 提出、識別番号 1-07000008 未完了報告
30	感染症および寄生虫症	B型肝炎	日本	男	74	2007/1/12	⑥不明	症例報告	当該製品	2007/3/2 提出、識別番号 1-06000235 未完了報告

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感染症発生症例一覧

31	感染症および寄生虫症	B型肝炎	日本	男	64	2007/1/11	③未回復	症例報告	当該製品	2007/2/22 提出、識別番号 1-06000229 未完了報告
32	感染症および寄生虫症	B型肝炎	日本	女	60	2007/1/11	⑤死亡	症例報告	当該製品	2007/3/20 提出、識別番号 1-06000262 未完了報告
33	感染症および寄生虫症	B型肝炎	日本	女	92	2006/12/20	②軽快	症例報告	当該製品	2007/1/24 提出、識別番号 1-06000211 未完了報告
34	感染症および寄生虫症	B型肝炎	日本	男	2	2006/12/18	③未回復	症例報告	当該製品	2007/2/6 提出、識別番号 1-06000216 未完了報告
35	感染症および寄生虫症	C型肝炎	日本	男	70	2006/12/14	③未回復	症例報告	当該製品	2007/1/10 提出、識別番号 1-06000209 未完了報告
36	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
37	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 (36番と同一症例)
38	感染症および寄生虫症	ブドウ球菌感染	日本	女	64	2006/12/11	⑤死亡	症例報告	当該製品	2007/1/24 提出、識別番号 1-06000212 未完了報告
39	感染症および寄生虫症	細菌感染	日本	男	64	2006/12/8	②軽快	症例報告	当該製品	2006/12/22 提出、識別番号 1-06000198 未完了報告
40	感染症および寄生虫症	B型肝炎	日本	女	68	2006/12/8	③未回復	症例報告	当該製品	2007/1/10 提出、識別番号 1-06000206 未完了報告
41	感染症および寄生虫症	B型肝炎	日本	女	68	2006/12/8	③未回復	症例報告	当該製品	2007/1/24提出、識別番号1-06000206 未完了報告 (40番と同一症例)
42	感染症および寄生虫症	C型肝炎	日本	女	59	2006/12/7	③未回復	症例報告	当該製品	2007/3/6 提出、識別番号 1-06000238 未完了報告
43	感染症および寄生虫症	C型肝炎	日本	男	44	2006/12/5	③未回復	症例報告	当該製品	2006/12/27 提出、識別番号 1-06000202 未完了報告
44	感染症および寄生虫症	サイトメガロウイルス感染	日本	女	1	2006/12/5	③未回復	症例報告	当該製品	2007/3/2提出、識別番号1-06000234 未完了報告 (26番と同一症例)
45	感染症および寄生虫症	B型肝炎	日本	男	94	2006/12/4	③未回復	症例報告	当該製品	2007/1/10 提出、識別番号 1-06000208 未完了報告
46	感染症および寄生虫症	B型肝炎	日本	男	94	2006/12/4	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-06000208 取り下げ (45番と同一症例)
47	感染症および寄生虫症	C型肝炎	日本	女	87	2006/12/1	②軽快	症例報告	当該製品	2006/12/25 提出、識別番号 1-06000199 未完了報告
48	感染症および寄生虫症	B型肝炎	日本	女	39	2006/11/29	③未回復	症例報告	当該製品	2007/1/5 提出、識別番号 1-06000204 未完了報告
49	感染症および寄生虫症	細菌感染	日本	男	85	2006/11/28	①回復	症例報告	当該製品	2006/12/15 提出、識別番号 1-06000190 未完了報告
50	感染症および寄生虫症	B型肝炎	日本	男	9	2006/11/27	⑥不明	症例報告	当該製品	2006/12/18 提出、識別番号 1-06000192 未完了報告
51	感染症および寄生虫症	C型肝炎	日本	男	82	2006/11/27	①回復	症例報告	当該製品	2006/12/18 提出、識別番号 1-06000193 未完了報告
52	感染症および寄生虫症	敗血症	日本	男	93	2006/11/27	⑤死亡	症例報告	当該製品	2006/12/22 提出、識別番号 1-06000194 未完了報告
53	感染症および寄生虫症	サイトメガロウイルス感染	日本	男	1	2006/11/15	③未回復	症例報告	当該製品	2006/12/18 提出、識別番号 1-06000191 未完了報告
54	感染症および寄生虫症	細菌感染	日本	男	85	2006/11/14	⑥不明	症例報告	当該製品	2006/11/30 提出、識別番号 1-06000185 未完了報告
55	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
56	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 (55番と同一症例)
57	感染症および寄生虫症	C型肝炎	日本	女	80	2006/10/30	⑥不明	症例報告	当該製品	2006/11/13 提出、識別番号 1-06000171 未完了報告
58	感染症および寄生虫症	細菌感染	日本	男	75	2006/10/27	①回復	症例報告	当該製品	2006/11/13 提出、識別番号 1-06000170 未完了報告
59	感染症および寄生虫症	C型肝炎	日本	男	42	2006/10/26	⑥不明	症例報告	当該製品	2006/12/22 提出、識別番号 1-06000197 未完了報告
60	感染症および寄生虫症	菌血症	日本	女	81	2006/10/25	①回復	症例報告	当該製品	2006/11/8 提出、識別番号 1-06000168 未完了報告
61	感染症および寄生虫症	マイコバクテリア感染	日本	女	3	2006/10/22	③未回復	症例報告	当該製品	2006/11/22 提出、識別番号 1-06000181 未完了報告

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感染症発生症例一覧

62	感染症および寄生虫症	細菌感染	日本	男	69	2006/10/21	①回復	症例報告	当該製品	2006/11/8 提出、識別番号 1-06000167 未完了報告
63	感染症および寄生虫症	C型肝炎	日本	女	94	2006/10/19	③未回復	症例報告	当該製品	2006/12/13 提出、識別番号 1-06000189 未完了報告
64	感染症および寄生虫症	菌血症	日本	男	92	2006/10/18	②軽快	症例報告	当該製品	2006/11/7 提出、識別番号 1-06000150 未完了報告
65	感染症および寄生虫症	B型肝炎	日本	女	42	2006/10/16	③未回復	症例報告	当該製品	2006/11/30 提出、識別番号 1-06000183 未完了報告
66	感染症および寄生虫症	B型肝炎	日本	女	13	2006/10/16	③未回復	症例報告	当該製品	2006/12/13 提出、識別番号 1-06000188 未完了報告
67	感染症および寄生虫症	C型肝炎	日本	男	75	2006/10/10	③未回復	症例報告	当該製品	2006/11/7 提出、識別番号 1-06000148 未完了報告
68	感染症および寄生虫症	C型肝炎	日本	女	64	2006/10/7	⑥不明	症例報告	当該製品	2007/3/9 提出、識別番号 1-06000242 未完了報告
69	感染症および寄生虫症	B型肝炎	日本	男	65	2006/10/3	③未回復	症例報告	当該製品	2006/11/1 提出、識別番号 1-06000146 未完了報告
70	感染症および寄生虫症	B型肝炎	日本	女	40	2006/10/3	③未回復	症例報告	当該製品	2006/11/1 提出、識別番号 1-06000147 未完了報告
71	感染症および寄生虫症	C型肝炎	日本	男	73	2006/9/27	②軽快	症例報告	当該製品	2007/2/6提出、識別番号1-06000141 取り下げ 第8回症例番号71は前回報告における第7回症例番号1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	63	2006/9/26	⑤死亡	症例報告	当該製品	2007/3/6 提出、識別番号 1-06000237 未完了報告
73	感染症および寄生虫症	B型肝炎	日本	男	65	2006/9/22	⑥不明	症例報告	当該製品	2006/11/6提出、識別番号1-06000136 未完了報告 第8回症例番号73は前回報告における第7回症例番号3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	C型肝炎	日本	男	77	2006/9/22	②軽快	症例報告	当該製品	2006/11/8 提出、識別番号 1-06000166 未完了報告
75	感染症および寄生虫症	C型肝炎	日本	女	47	2006/9/14	③未回復	症例報告	当該製品	2007/1/18 提出、識別番号 1-06000210 未完了報告
76	感染症および寄生虫症	B型肝炎	日本	男	53	2006/7/27	②軽快	症例報告	当該製品	2006/12/27 提出、識別番号 1-06000200 未完了報告
77	感染症および寄生虫症	B型肝炎	日本	女	52	2006/7/20	③未回復	症例報告	当該製品	2006/11/2提出、識別番号1-06000122 未完了報告 第8回症例番号77は前回報告における第7回症例番号27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	敗血症	日本	男	70	2006/5/1	⑤死亡	症例報告	当該製品	2006/12/5提出、識別番号1-06000023 完了報告 第8回症例番号78は前回報告における第7回症例番号69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	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78番と同一症例) 第8回症例番号79は前回報告における第7回症例番号69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	56	2006/4/10	⑥不明	症例報告	当該製品	2007/2/26提出、識別番号1-06000015 取り下げ 第8回症例番号80は前回報告における第7回症例番号87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	C型肝炎	日本	男	92	2006/4/5	⑥不明	症例報告	当該製品	2007/2/26提出、識別番号1-06000030 取り下げ 第8回症例番号81は前回報告における第7回症例番号88において報告したものの追加報告
82	感染症および寄生虫症	B型肝炎	日本	女	61	2006/3/31	③未回復	症例報告	当該製品	2007/4/13 提出、識別番号 1-07000009 未完了報告

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83	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8回症例番号83は前々回報告における第6回症例番号4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	Ｃ型肝炎	日本	女	92	2006/3/14	③未回復	症例報告	当該製品	2007/1/31提出、識別番号1-06000001　完了報告 第8回症例番号84は前々回報告における第6回症例番号8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	細菌感染	日本	男	34	2006/2/11	①回復	症例報告	当該製品	2007/3/29提出、識別番号1-05000262　完了報告 第8回症例番号85は前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
86	感染症および寄生虫症	ブドウ球菌性敗血症	日本	女	71	2006/2/7	①回復	症例報告	当該製品	2007/3/29提出、識別番号1-05000263　完了報告 第8回症例番号86は前々回報告における第6回症例番号19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	男	54	2006/1/30	①回復	症例報告	当該製品	2007/1/31提出、識別番号1-05000251　完了報告 第8回症例番号87は前々回報告における第6回症例番号21において報告したものの追加報告
88	感染症および寄生虫症	ブドウ球菌感染	日本	女	73	2006/1/25	⑤死亡	症例報告	当該製品	2007/3/29提出、識別番号1-05000253　完了報告 第8回症例番号88は前々回報告における第6回症例番号26において報告したものの追加報告
89	感染症および寄生虫症	Ｃ型肝炎	日本	男	63	2006/1/20	③未回復	症例報告	当該製品	2007/1/31提出、識別番号1-05000269　完了報告 第8回症例番号89は前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
90	感染症および寄生虫症	Ｅ型肝炎	日本	男	58	2006/1/16	⑥不明	症例報告	当該製品	2006/12/28提出、識別番号1-05000244　完了報告 第8回症例番号90は前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
91	感染症および寄生虫症	Ｂ型肝炎	日本	女	52	2005/12/1	①回復	症例報告	当該製品	2007/1/31提出、識別番号1-05000259　完了報告 第8回症例番号91は前々回報告における第6回症例番号57において報告したものの追加報告
92	感染症および寄生虫症	Ｂ型肝炎	日本	女	54	2005/11/2	③未回復	症例報告	当該製品	2007/2/26提出、識別番号1-06000117　取り下げ 第8回症例番号92は前回報告における第7回症例番号102において報告したものの追加報告
93	感染症および寄生虫症	Ｂ型肝炎	日本	女	89	2005/6/29	①回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000092　完了報告 第8回症例番号93は前々々回報告における第5回症例番号67において報告したものの追加報告
94	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8回症例番号94は前々々回報告における第5回症例番号89において報告したものの追加報告

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95	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8回症例番号95は前々々回報告における第5回症例番号94において報告したものの追加報告
96	感染症および寄生虫症	C型肝炎	日本	男	41	2005/5/18	②軽快	症例報告	当該製品	2006/12/21提出、識別番号1-05000044　完了報告 第8回症例番号96は前々々回報告における第5回症例番号95において報告したものの追加報告
97	感染症および寄生虫症	B型肝炎	日本	女	57	2005/4/22	②軽快	症例報告	当該製品	2007/3/30提出、識別番号1-05000046　完了報告 第8回症例番号97は前々々回報告における第5回症例番号104において報告したものの追加報告
98	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8回症例番号98は前々々回報告における第5回症例番号105、106において報告したものの追加報告
99	感染症および寄生虫症	C型肝炎	日本	女	10	2005/4/19	①回復	症例報告	当該製品	2006/12/21提出、識別番号1-05000033　完了報告 第8回症例番号99は前々々回報告における第5回症例番号111において報告したものの追加報告
100	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8回症例番号100は前々々回報告における第5回症例番号115において報告したものの追加報告
101	感染症および寄生虫症	C型肝炎	日本	女	91	2005/4/7	②軽快	症例報告	当該製品	2006/12/21提出、識別番号1-05000019　完了報告 第8回症例番号101は前々々々回報告における第4回症例番号1において報告したものの追加報告
102	感染症および寄生虫症	C型肝炎	日本	男	42	2005/3/21	⑥不明	症例報告	当該製品	2006/12/21提出、識別番号1-05000042　完了報告 第8回症例番号102は前々々回報告における第5回症例番号121において報告したものの追加報告
103	感染症および寄生虫症	C型肝炎	日本	男	7	2005/3/18	⑥不明	症例報告	当該製品	2006/12/21提出、識別番号1-05000030　完了報告 第8回症例番号103は前々々回報告における第5回症例番号122、123において報告したものの追加報告
104	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8回症例番号104は前々々々回報告における第4回症例番号22において報告したものの追加報告
105	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8回症例番号105は前々々々回報告における第4回症例番号23において報告したものの追加報告
106	感染症および寄生虫症	B型肝炎	日本	女	89	2005/2/8	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-05000065　完了報告 第8回症例番号106は前々々回報告における第5回症例番号129において報告したものの追加報告

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107	感染症および寄生虫症	C型肝炎	日本	男	23	2005/2/7	⑥不明	症例報告	当該製品	2007/3/19提出、識別番号1-04000327　完了報告第8回症例番号107は前々々々回報告における第4回症例番号25において報告したものの追加報告
108	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　完了報告第8回症例番号108は前々々々回報告における第4回症例番号35において報告したものの追加報告
109	感染症および寄生虫症	B型肝炎	日本	女	62	2004/12/21	③未回復	症例報告	当該製品	2007/2/23提出、識別番号1-04000285　完了報告第8回症例番号109は前々々々回報告における第4回症例番号50において報告したものの追加報告
110	感染症および寄生虫症	C型肝炎	日本	男	76	2004/12/14	③未回復	症例報告	当該製品	2007/2/19提出、識別番号1-04000279　完了報告第8回症例番号110は前々々々回報告における第4回症例番号53において報告したものの追加報告
111	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8回症例番号111は前々々々回報告における第4回症例番号57において報告したものの追加報告
112	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8回症例番号112は前々々々回報告における第4回症例番号59において報告したものの追加報告
113	感染症および寄生虫症	B型肝炎	日本	女	59	2004/11/29	①回復	症例報告	当該製品	2007/1/23提出、識別番号1-04000281　完了報告第8回症例番号113は前々々々回報告における第4回症例番号63において報告したものの追加報告
114	感染症および寄生虫症	B型肝炎	日本	男	68	2004/11/18	⑤死亡	症例報告	当該製品	2007/2/27提出、識別番号1-04000256　完了報告第8回症例番号114は前々々々回報告における第4回症例番号70において報告したものの追加報告
115	感染症および寄生虫症	C型肝炎	日本	男	57	2004/11/10	②軽快	症例報告	当該製品	2007/4/3提出、識別番号1-04000254　完了報告第8回症例番号115は前々々々回報告における第4回症例番号74、75及び前々々回報告における第5回症例番号145、146において報告したものの追加報告
116	感染症および寄生虫症	B型肝炎	日本	男	65	2004/10/25	②軽快	症例報告	当該製品	2007/2/23提出、識別番号1-04000249　完了報告第8回症例番号116は前々々々回報告における第4回症例番号81、82において報告したものの追加報告
117	感染症および寄生虫症	C型肝炎	日本	女	88	2004/10/21	⑥不明	症例報告	当該製品	2007/3/16提出、識別番号1-04000235　完了報告第8回症例番号117は前々々々回報告における第4回症例番号83において報告したものの追加報告
118	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8回症例番号118は前々々々回報告における第4回症例番号87、88、89、90において報告したものの追加報告

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119	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 (118番と同一症例) 第8回症例番号118は前々々々回報告における第4回症例番号87、88、89、90において報告したものの追加報告
120	感染症および寄生虫症	C型肝炎	日本	女	75	2004/10/14	⑥不明	症例報告	当該製品	2007/3/30提出、識別番号1-05000084　完了報告 第8回症例番号120は前々々回報告における第5回症例番号153において報告したものの追加報告
121	感染症および寄生虫症	B型肝炎	日本	男	52	2004/10/12	②軽快	症例報告	当該製品	2007/3/19提出、識別番号1-04000314　完了報告 第8回症例番号121は前々々々回報告における第4回症例番号92において報告したものの追加報告
122	感染症および寄生虫症	B型肝炎	日本	男	72	2004/10/8	⑤死亡	症例報告	当該製品	2007/2/27 提出、識別番号 1-04000224 完了報告
123	感染症および寄生虫症	C型肝炎	日本	男	89	2004/10/6	⑥不明	症例報告	当該製品	2006/12/21提出、識別番号1-05000020　完了報告 第8回症例番号123は前々々々回報告における第4回症例番号95において報告したものの追加報告
124	感染症および寄生虫症	B型肝炎	日本	男	80	2004/10/4	①回復	症例報告	当該製品	2007/2/19提出、識別番号1-04000251　完了報告 第8回症例番号124は前々々々回報告における第4回症例番号97において報告したものの追加報告
125	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
126	感染症および寄生虫症	B型肝炎	日本	男	49	2004/9/14	⑥不明	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000206 完了報告
127	感染症および寄生虫症	B型肝炎	日本	女	67	2004/9/13	⑥不明	症例報告	当該製品	2007/2/19提出、識別番号1-04000250　完了報告 第8回症例番号127は前々々々回報告における第4回症例番号102において報告したものの追加報告
128	感染症および寄生虫症	C型肝炎	日本	女	48	2004/9/9	②軽快	症例報告	当該製品	2007/2/13提出、識別番号1-04000230　完了報告 第8回症例番号128は前々々々回報告における第4回症例番号103において報告したものの追加報告
129	感染症および寄生虫症	C型肝炎	日本	男	90	2004/9/2	⑤死亡	症例報告	当該製品	2007/2/13 提出、識別番号 1-04000194 完了報告
130	感染症および寄生虫症	B型肝炎	日本	女	57	2004/8/20	②軽快	症例報告	当該製品	2007/2/19 提出、識別番号 1-04000215 完了報告
131	感染症および寄生虫症	C型肝炎	日本	男	69	2004/8/17	②軽快	症例報告	当該製品	2007/3/15 提出、識別番号 1-04000170 完了報告
132	感染症および寄生虫症	C型肝炎	日本	男	76	2004/8/17	③未回復	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000173 完了報告
133	感染症および寄生虫症	B型肝炎	日本	女	43	2004/8/16	②軽快	症例報告	当該製品	2007/1/23 提出、識別番号 1-04000209 完了報告
134	感染症および寄生虫症	B型肝炎	日本	女	55	2004/8/10	⑥不明	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000205 完了報告
135	感染症および寄生虫症	C型肝炎	日本	男	57	2004/8/5	③未回復	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000164 完了報告
136	感染症および寄生虫症	B型肝炎	日本	女	59	2004/7/30	⑥不明	症例報告	当該製品	2007/1/23提出、識別番号1-04000231　完了報告 第8回症例番号136は前々々々回報告における第4回症例番号107において報告したものの追加報告
137	感染症および寄生虫症	C型肝炎	日本	女	76	2004/7/22	⑥不明	症例報告	当該製品	2007/3/16 提出、識別番号 1-04000140 完了報告
138	感染症および寄生虫症	B型肝炎	日本	男	58	2004/6/29	⑥不明	症例報告	当該製品	2007/2/13 提出、識別番号 1-04000133 完了報告

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	139	感染症および寄生虫症	C型肝炎	日本	男	45	2004/6/11	⑥不明	症例報告	当該製品	2007/2/27 提出、識別番号 1-04000103 完了報告
	140	感染症および寄生虫症	B型肝炎	日本	男	62	2004/5/14	⑥不明	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000124 完了報告
	141	感染症および寄生虫症	C型肝炎	日本	男	59	2004/4/16	③未回復	症例報告	当該製品	2007/2/13 提出、識別番号 1-04000039 完了報告
	142	感染症および寄生虫症	B型肝炎	日本	男	71	2004/2/16	①回復	症例報告	当該製品	2006/11/7提出、識別番号1-06000149 未完了報告
	143	感染症および寄生虫症	B型肝炎	日本	男	71	2004/2/16	①回復	症例報告	当該製品	2006/12/26提出、識別番号1-06000149 未完了報告 (142番と同一症例)
	144	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8回症例番号144は前々回報告における第6回症例番号186において報告したものの追加報告
	145	感染症および寄生虫症	伝染性紅斑	日本	女	55	2003/8/15	①回復	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000177 完了報告
	146	感染症および寄生虫症	B型肝炎	日本	男	62	2003/6/18	⑥不明	症例報告	当該製品	2007/2/19 提出、識別番号 1-04000208 完了報告
	147	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8回症例番号147は前々々々々回報告における第3回症例番号50及び前々々々回報告における第4回症例番号148において報告したものの追加報告
	148	感染症および寄生虫症	サイトメガロウイルス感染	日本	男	7	2002/12/22	②軽快	症例報告	当該製品	2007/2/13 提出、識別番号 1-04000105 完了報告
	149	感染症および寄生虫症	B型肝炎	日本	男	50	2002/6/13	①回復	症例報告	当該製品	2007/2/23 提出、識別番号 1-04000220 完了報告
	150	感染症および寄生虫症	E型肝炎	日本	女	69	2002/5/14	③未回復	症例報告	当該製品	2007/3/30提出、識別番号1-04000312 完了報告 第8回症例番号150は前々々々回報告における第4回症例番号149において報告したものの追加報告
	151	感染症および寄生虫症	C型肝炎	日本	女	30	2001/8/2	①回復	症例報告	当該製品	2007/3/19提出、識別番号1-04000336 完了報告 第8回症例番号151は前々々々回報告における第4回症例番号150において報告したものの追加報告
第7回	1	感染症および寄生虫症	C型肝炎	日本	男	73	2006/9/27	②軽快	症例報告	当該製品	2006/10/24 提出、識別番号 1-06000141 未完了報告
	3	感染症および寄生虫症	B型肝炎	日本	男	66	2006/9/22	⑥不明	症例報告	当該製品	2006/10/13 提出、識別番号 1-06000136 未完了報告
	27	感染症および寄生虫症	B型肝炎	日本	女	52	2006/7/20	③未回復	症例報告	当該製品	2006/9/22 提出、識別番号 1-06000122 未完了報告
	69	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
	87	感染症および寄生虫症	B型肝炎	日本	男	56	2006/4/10	③未回復	症例報告	当該製品	2006/5/2 提出、識別番号 1-06000015 未完了報告
	88	感染症および寄生虫症	C型肝炎	日本	男	92	2006/4/5	⑥不明	症例報告	当該製品	2006/5/25 提出、識別番号 1-06000030 未完了報告
	102	感染症および寄生虫症	B型肝炎	日本	女	54	2005/11/2	③未回復	症例報告	当該製品	2006/9/14 提出、識別番号 1-06000117 未完了報告
第6回	4	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
	8	感染症および寄生虫症	C型肝炎	日本	女	92	2006/3/14	③未回復	症例報告	当該製品	2006/4/6 提出、識別番号 1-06000001 未完了報告
	17	感染症および寄生虫症	細菌感染	日本	男	34	2006/2/11	②軽快	症例報告	当該製品	2006/3/1 提出、識別番号 1-05000262 未完了報告
	19	感染症および寄生虫症	ブドウ球菌性創感染	日本	女	71	2006/2/7	③未回復	症例報告	当該製品	2006/3/1 提出、識別番号 1-05000263 未完了報告
	21	感染症および寄生虫症	B型肝炎	日本	男	54	2006/1/30	③未回復	症例報告	当該製品	2006/2/15 提出、識別番号 1-05000251 未完了報告
	26	感染症および寄生虫症	ブドウ球菌感染	日本	女	73	2006/1/25	⑤死亡	症例報告	当該製品	2006/2/15 提出、識別番号 1-05000253 未完了報告
	31	感染症および寄生虫症	C型肝炎	日本	男	69	2006/1/20	③未回復	症例報告	当該製品	2006/3/15 提出、識別番号 1-05000269 未完了報告

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	34	感染症および寄生虫症	E型肝炎	日本	男	58	2006/1/16	⑥不明	症例報告	当該製品	2006/2/2 提出、識別番号 1-05000244 未完了報告
	57	感染症および寄生虫症	B型肝炎	日本	女	52	2005/12/1	①回復	症例報告	当該製品	2006/2/24 提出、識別番号 1-05000259 未完了報告
	186	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	67	感染症および寄生虫症	B型肝炎	日本	女	89	2005/06/29	③未回復	症例報告	当該製品	2005/07/26 提出、識別番号 1-05000092 取り下げ
	89	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 取り下げ
	94	感染症および寄生虫症	B型肝炎	日本	女	23	2005/05/19	⑥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
	95	感染症および寄生虫症	C型肝炎	日本	男	41	2005/05/18	③未回復	症例報告	当該製品	2005/06/07 提出、識別番号 1-05000044 未完了報告
	104	感染症および寄生虫症	B型肝炎	日本	女	57	2005/04/22	③未回復	症例報告	当該製品	2005/06/14 提出、識別番号 1-05000046 未完了報告
	105	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
	106	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
	111	感染症および寄生虫症	C型肝炎	日本	女	10	2005/04/19	①回復	症例報告	当該製品	2005/05/23 提出、識別番号 1-05000033 未完了報告
	115	感染症および寄生虫症	B型肝炎	日本	女	65	2005/04/15	④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
	121	感染症および寄生虫症	C型肝炎	日本	男	42	2005/03/21	③未回復	症例報告	当該製品	2005/06/07 提出、識別番号 1-05000042 未完了報告
	122	感染症および寄生虫症	C型肝炎	日本	男	7	2005/03/18	③未回復	症例報告	当該製品	2005/05/13 提出、識別番号 1-05000030 未完了報告
	123	感染症および寄生虫症	C型肝炎	日本	男	7	2005/03/18	③未回復	症例報告	当該製品	2005/05/13 提出、識別番号 1-05000030 未完了報告
	129	感染症および寄生虫症	B型肝炎	日本	女	89	2005/02/08	⑥不明	症例報告	当該製品	2005/06/29 提出、識別番号 1-05000065 未完了報告
	145	感染症および寄生虫症	C型肝炎	日本	男	57	2004/11/10	②軽快	症例報告	当該製品	2005/06/15 提出、識別番号 1-04000254 完了報告
	146	感染症および寄生虫症	C型肝炎	日本	男	57	2004/11/10	②軽快	症例報告	当該製品	2005/06/15 提出、識別番号 1-04000254 完了報告
	153	感染症および寄生虫症	C型肝炎	日本	女	75	2004/10/14	⑥不明	症例報告	当該製品	2005/07/19 提出、識別番号 1-05000084 完了報告
第4回	1	感染症および寄生虫症	C型肝炎	日本	女	91	2005/04/07	①軽快	症例報告	当該製品	2005/04/26 提出、識別番号 1-05000019 未完了報告
	22	感染症および寄生虫症	C型肝炎	日本	男	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
	23	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
	25	感染症および寄生虫症	C型肝炎	日本	男	23	2005/02/07	⑧不明	症例報告	当該製品	2005/03/08 提出、識別番号 1-04000327 未完了報告
	35	感染症および寄生虫症	C型肝炎	日本	女	3	2005/01/19	③未回復	症例報告	当該製品	2005/02/15 提出、識別番号 1-04000313 未完了報告
	50	感染症および寄生虫症	B型肝炎	日本	女	62	2004/12/27	③未回復	症例報告	当該製品	2005/01/07 提出、識別番号 1-04000285 未完了報告
	53	感染症および寄生虫症	C型肝炎	日本	男	76	2004/12/14	③未回復	症例報告	当該製品	2005/01/04 提出、識別番号 1-04000279 未完了報告
	57	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
	59	感染症および寄生虫症	C型肝炎	日本	男	84	2004/12/06	③未回復	症例報告	当該製品	2005/02/18 提出、識別番号 1-04000315 未完了報告
	63	感染症および寄生虫症	B型肝炎	日本	女	59	2004/11/29	①回復	症例報告	当該製品	2005/01/04 提出、識別番号 1-04000281 未完了報告
	70	感染症および寄生虫症	B型肝炎	日本	男	68	2004/11/18	③未回復	症例報告	当該製品	2004/12/07 提出、識別番号 1-04000256 未完了報告
	74	感染症および寄生虫症	C型肝炎	日本	男	56	2004/11/10	⑧不明	症例報告	当該製品	2004/12/02 提出、識別番号 1-04000254 未完了報告
	75	感染症および寄生虫症	C型肝炎	日本	男	56	2004/11/10	⑧不明	症例報告	当該製品	2004/12/02 提出、識別番号 1-04000254 未完了報告
	81	感染症および寄生虫症	B型肝炎	日本	男	65	2004/10/25	①軽快	症例報告	当該製品	2004/12/01 提出、識別番号 1-04000249 未完了報告
	82	感染症および寄生虫症	B型肝炎	日本	男	65	2004/10/25	①軽快	症例報告	当該製品	2004/12/01 提出、識別番号 1-04000249 未完了報告
	83	感染症および寄生虫症	C型肝炎	日本	女	88	2004/10/21	⑧不明	症例報告	当該製品	2004/11/10 提出、識別番号 1-04000235 未完了報告
	87	感染症および寄生虫症	ヘルペスウイルス肝炎	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

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	88	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
	89	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
	90	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
	92	感染症および寄生虫症	B型肝炎	日本	男	52	2004/10/12	①軽快	症例報告	当該製品	2005/02/15 提出、識別番号 1-04000314 未完了報告
	95	感染症および寄生虫症	C型肝炎	日本	男	89	2004/10/06	⑧不明	症例報告	当該製品	2005/04/26 提出、識別番号 1-05000020 未完了報告
	97	感染症および寄生虫症	B型肝炎	日本	男	80	2004/10/04	⑧不明	症例報告	当該製品	2004/12/02 提出、識別番号 1-04000251 未完了報告
	102	感染症および寄生虫症	B型肝炎	日本	女	67	2004/09/13	⑧不明	症例報告	当該製品	2004/12/02 提出、識別番号 1-04000250 未完了報告
	103	感染症および寄生虫症	C型肝炎	日本	女	48	2004/09/09	①軽快	症例報告	当該製品	2004/11/02 提出、識別番号 1-04000230 未完了報告
	107	感染症および寄生虫症	B型肝炎	日本	女	59	2004/07/30	⑧不明	症例報告	当該製品	2004/11/02 提出、識別番号 1-04000231 未完了報告
	148	感染症および寄生虫症	B型肝炎	日本	女	1	2003/01/10	①軽快	症例報告	当該製品	2005/02/03 提出、識別番号 1-04000072 完了報告
	149	感染症および寄生虫症	E型肝炎	日本	女	69	2002/05/14	③未回復	症例報告	当該製品	2005/02/14 提出、識別番号 1-04000312 未完了報告
	150	感染症および寄生虫症	C型肝炎	日本	女	30	2001/08/02	①回復	症例報告	当該製品	2005/03/15 提出、識別番号 1-04000336 未完了報告
第3回	50	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 データなし

70060	2007/06/15	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液

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感染症発生症例一覧

	番号	感染症の種類（MedDRA 9.1）		発現国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第8回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	自発報告	外国製品 1)	識別番号 6000026 第2回報告 平成18年12月27日 （完了報告）
	1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	自発報告	外国製品 1)	識別番号 6000026 第1回報告 平成18年12月8日 （未完了報告）

1)販売名：Kybernin P（アンチトロンビンIII製剤、ZLB BEHRING）

70063	2007/06/18	化学及血清療法研究所	乾燥濃縮人アンチトロンビンIII	アンチトロンビンIII

別紙様式第4

感染症発生症例一覧

	番号	感染症の種類		発生国	性別	年齢	発現時期	転帰	出典	区分	備考
		器官別大分類	基本語								
第8回	1	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3-06000034 報告書提出日2007年2月23日
	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3-06000034 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3-06000034 完了報告書提出日2007年3月30日
第7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

70075	2007/07/26	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

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8回	1	感染症および寄生虫症	C型肝炎	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告書提出日2006年12月20日
	1	臨床検査	C型肝炎抗体陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告書提出日2006年12月20日
	1	臨床検査	C型肝炎RNA陽性	ドイツ	女	41	2006/11/21	不明	症例報告	外国製品	識別番号3-06000029 報告書提出日2006年12月20日
第7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

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70077	2007/07/26	CSLベーリング	乾燥濃縮人アンチトロンビンⅢ	乾燥濃縮人アンチトロンビンⅢ

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	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1は前々々々々回報告における第4回症例番号1において報告したものの追加報告
第4回	1	感染症および寄生虫症	B型肝炎	日本	男	51	2004/09/30	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

70081	2007/07/27	日本赤十字社	洗浄人赤血球浮遊液	洗浄人赤血球浮遊液

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	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1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
	2	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2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
	3	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3は前々々々々回報告における第4回症例番号3及び前々々々々々回報告における第3回症例番号3において報告したものの追加報告
第5回	3	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
	4	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
第4回	3	感染症および寄生虫症	B型肝炎	日本	女	1	2003/01/10	①軽快	症例報告	当該製品	2005/02/03 提出、識別番号　1-04000072 完了報告
第3回	3	感染症および寄生虫症	B型肝炎	日本	女	1	2003/01/10	①軽快	症例報告	当該製品	2005/02/03 提出、識別番号　1-04000072 完了報告

70078	2007/07/27	日本赤十字社	白血球除去人赤血球浮遊液	白血球除去人赤血球浮遊液

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	C型肝炎	日本	女	95	2007/5/7	③未回復	症例報告	当該製品	2007/5/28 提出、識別番号 1-07000028 未完了報告
	2	感染症および寄生虫症	B型肝炎	日本	男	84	2007/4/25	⑥不明	症例報告	当該製品	2007/5/25 提出、識別番号 1-07000027 未完了報告
	3	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 第9回症例番号3は前回報告における第8回症例番号1において報告したものの追加報告
	4	感染症および寄生虫症	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
	5	感染症および寄生虫症	B型肝炎	日本	女	60	2007/1/11	⑤死亡	症例報告	当該製品	2007/5/21提出、識別番号1-06000262 取り下げ 第9回症例番号5は前回報告における第8回症例番号32において報告したものの追加報告
	6	感染症および寄生虫症	B型肝炎	日本	女	80	2006/8/21	①回復	症例報告	当該製品	2007/5/15提出、識別番号1-06000116 完了報告 第9回症例番号6は前々回報告における第7回症例番号10において報告したものの追加報告
	7	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7は前々回報告における第7回症例番号63において報告したものの追加報告
	8	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8は前々回報告における第7回症例番号100において報告したものの追加報告
	9	感染症および寄生虫症	C型肝炎	日本	女	78	2005/12/7	②軽快	症例報告	当該製品	2007/5/17提出、識別番号1-05000223 完了報告 第9回症例番号9は前々々回報告における第6回症例番号52において報告したものの追加報告
	10	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10は前々々回報告における第6回症例番号58において報告したものの追加報告
	11	感染症および寄生虫症	B型肝炎	日本	女	2	2005/10/25	②軽快	症例報告	当該製品	2007/5/10提出、識別番号1-05000191 完了報告 第9回症例番号11は前々々回報告における第6回症例番号72において報告したものの追加報告
	12	感染症および寄生虫症	B型肝炎	日本	女	60	2005/10/7	⑥不明	症例報告	当該製品	2007/5/17提出、識別番号1-05000218 完了報告 第9回症例番号12は前々々回報告における第6回症例番号80において報告したものの追加報告
	13	感染症および寄生虫症	B型肝炎	日本	女	59	2005/9/29	⑥不明	症例報告	当該製品	2007/5/10提出、識別番号1-05000203 完了報告 第9回症例番号13は前々々回報告における第6回症例番号85において報告したものの追加報告

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別紙様式第4

感染症発生症例一覧

	14	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3は前々々回報告における第6回症例番号86において報告したものの追加報告
	15	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15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
	16	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16は前々々々回報告における第5回症例番号58において報告したものの追加報告
	17	感染症および寄生虫症	C型肝炎	日本	女	75	2005/6/1	③未回復	症例報告	当該製品	2007/5/22提出、識別番号1-05000052　完了報告　第9回症例番号17は前々々々回報告における第5回症例番号81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	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18は前々々々回報告における第5回症例番号107及び108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	男	59	2005/2/18	③未回復	症例報告	当該製品	2007/5/23提出、識別番号1-04000330　完了報告　第9回症例番号19は前々々々々回報告における第4回症例番号19において報告したものの追加報告
	20	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20は前々々々回報告における第4回症例番号43において報告したものの追加報告
	21	感染症および寄生虫症	B型肝炎	日本	男	92	2004/3/19	②軽快	症例報告	当該製品	2007/5/22提出、識別番号1-05000023　完了報告　第9回症例番号21は前々々々回報告における第5回症例番号171において報告したものの追加報告
第8回	1	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
	32	感染症および寄生虫症	B型肝炎	日本	女	60	2007/1/11	⑤死亡	症例報告	当該製品	2007/3/20 提出、識別番号 1-06000262 未完了報告
	10	感染症および寄生虫症	B型肝炎	日本	女	80	2006/8/21	③未回復	症例報告	当該製品	2006/9/14 提出、識別番号 1-06000116 未完了報告
第7回	63	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
	100	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	52	感染症および寄生虫症	C型肝炎	日本	女	78	2005/12/7	③未回復	症例報告	当該製品	2005/12/22 提出、識別番号 1-05000223 未完了報告
	58	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 1-0500022 未完了報告
	72	感染症および寄生虫症	B型肝炎	日本	女	2	2005/10/25	③未回復	症例報告	当該製品	2005/11/14 提出、識別番号 1-0500019 未完了報告
	80	感染症および寄生虫症	B型肝炎	日本	女	60	2005/10/7	③未回復	症例報告	当該製品	2005/12/20 提出、識別番号 1-0500021 未完了報告
	85	感染症および寄生虫症	B型肝炎	日本	女	59	2005/9/29	⑥不明	症例報告	当該製品	2005/11/28 提出、識別番号 1-0500020 未完了報告
	86	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 1-0500018 未完了報告
	46	感染症および寄生虫症	B型肝炎	日本	男	28	2005/07/15	②軽快	症例報告	当該製品	2005/08/09 提出、識別番号 1-0500011 未完了報告

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別紙様式第4

感染症発生症例一覧

第5回	58	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 1-0500010(未完了報告
	81	感染症および寄生虫症	C型肝炎	日本	女	75	2005/06/01	③未回復	症例報告	当該製品	2005/06/17 提出、識別番号 1-05000052 未完了報告
	107	感染症および寄生虫症	B型肝炎	日本	男	53	2005/04/21	⑤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
	108	感染症および寄生虫症	B型肝炎	日本	男	53	2005/04/21	⑤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
	171	感染症および寄生虫症	B型肝炎	日本	男	92	2004/03/19	③未回復	症例報告	当該製品	2005/04/28 提出、識別番号 1-05000023 未完了報告
第4回	19	感染症および寄生虫症	B型肝炎	日本	男	59	2005/02/18	③未回復	症例報告	当該製品	2005/03/10 提出、識別番号 1-04000330 未完了報告
	43	感染症および寄生虫症	B型肝炎	日本	男	22	2005/01/08	③未回復	症例報告	当該製品	2005/01/26 提出、識別番号 1-04000296 未完了報告

70079	2007/07/27	日本赤十字社	人赤血球濃厚液	人赤血球濃厚液

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第８回	8-1	臨床検査	Ｃ型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	自発報告	当該製品	識別番号：07000005 報告日：2007年4月9日 MedDRA: Version (9.1)
	8-1	臨床検査	Ｃ型肝炎抗体陽性	日本	男	11歳	2007/3/8	未回復	自発報告	当該製品	識別番号：07000005 報告日：2007年4月27日 2007年4月9日に提出した症例番号8-1の追加報告 MedDRA: Version (9.1)
第７回	該当なし										
第６回	5-1	臨床検査	ＨＩＶ検査陽性	韓国	男性	5歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2006年2月6日 第6回症例番号5-1は前回報告における第5回症例番号5-1において報告したものの取り下げ報告　MedDRA: Version (8.0)
	6-1	臨床検査	Ｂ型肝炎抗原陽性	アメリカ	男	66歳	2005/12/9	未回復	自発報告	当該製品	識別番号：05000495 報告日：2006年2月2日 MedDRA: Version (8.1)
第５回	5-1	臨床検査	ＨＩＶ検査陽性	韓国	男性	5歳	2004/9/15	未回復	症例報告	当該製品	識別番号：05000406 報告日：2005年8月18日 MedDRA: Version (8.0)
第４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３回	3-1	臨床検査	Ｃ型肝炎陽性	米国	男性	14 歳	2001/11/30	不明	症例報告	当該製品	識別番号：04000072 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
	3-2	臨床検査	Ｃ型肝炎陽性	米国	男性	10 歳	2002/9/11	不明	症例報告	当該製品	識別番号：04000073 報告日：2004 年 12 月 13 日 MedDRA: Version(7.1)
第２回	2-1	臨床検査	Ａ型肝炎抗体陽性	フランス	不明	50 歳	不明	不明	症例報告	当該製品	識別番号：03000021 報告日：2004 年 2 月 18 日 MedDRA: Version(6.1)

注）第１回は該当なし。

70084	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ルリオクトコグ　アルファ(遺伝子組換え)
70085	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	人血清アルブミン
70086	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)
70087	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造用)
70088	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	アプロチニン
70089	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	ウシ血清アルブミン
70090	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	培養補助剤（抗第Ⅷ因子モノクローナル抗体製造用-1)
70091	2007/07/31	バクスター	ルリオクトコグ　アルファ(遺伝子組換え)	培養補助剤（抗第Ⅷ因子モノクローナル抗体製造用-2)