

薬事・食品衛生審議会
平成21年度 第1回 血液事業部会運営委員会

議 事 次 第

日時：平成21年5月14日（木）

15:00～17:00

場所：航空会館（7F）

702・703会議室

東京都港区新橋1-18-1

議題：

1. 委員長の選出及び委員長代理の指名
2. 議事要旨の確認
3. 感染症定期報告について
4. 血液製剤に関する報告事項について
5. 日本赤十字社からの報告事項について
6. その他

配付資料：

- 資 料 1 平成20年度第4回血液事業部会運営委員会議事要旨（案）
- 資 料 2 感染症定期報告について
- 資料3-1 供血者からの遡及調査の進捗状況について
- 資料3-2 血液製剤に関する報告事項について
- 資料3-3 献血件数及びHIV抗体・核酸増幅検査陽性件数
- 資 料 4 NATコントロールサーベイの結果について
- 資 料 5 遺伝子組換えアルブミン製剤供給停止に伴う需給計画への影響について
- （参考）遺伝子組換え人血清アルブミン製剤「メドウェイ注5%」製造販売承認の取下げおよび「メドウェイ注5%」「メドウェイ注25%」の自主回収について（平成21年3月24日公表）
- 資料6-1 日本赤十字社血液事業本部組織の変更について
- 資料6-2 「血液事業における新型インフルエンザ対策ガイドライン（暫定版）」の作成について

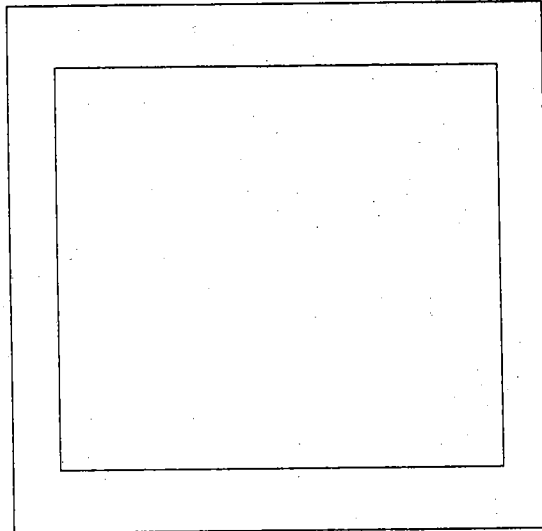
- 資料7-1 フィブリノゲン製剤納入先医療機関の追加調査について（平成21年4月24日公表）
- 資料7-2 フィブリノゲン製剤等に関する相談窓口について（平成21年3月30日公表）
- 資料7-3 C型肝炎訴訟の和解について（平成21年1月28日公表）
- 資料7-4 C型肝炎救済特措法の給付金に対する製薬企業の負担割合について（告示の制定）（平成21年4月10日公表）
- 資料7-5 田辺三菱製薬株式会社等における個人情報の開示請求への対応等について（平成21年2月26日公表）
- 資料7-6 田辺三菱製薬株式会社におけるフィブリノゲン製剤に係る418症例報告調査プロジェクトチーム活動状況等の報告について（平成21年1月27日公表）
- 資料7-7 血液凝固因子製剤の納入先医療機関の調査について（平成20年4月24日公表）
- 資料7-8 厚生労働省科学研究費補助金厚生労働科学特別研究事業
フィブリノゲン製剤等の納入医療機関における製剤の使用実態及び当該製剤を使用された患者における肝炎ウイルス感染等の実態に関する研究について

薬事・食品衛生審議会薬事分科会 血液事業部会運営委員会 委員名簿

速記

委員
長
席

大平委員
岡田委員
佐川委員
高橋委員
花井委員
山口委員



(日本赤十字社)

血液課長
血液対策課長
血液対策課長
血液課長
血液対策企画官
血液対策課長
血液課長
血液対策課長
血液課長
血液対策課長
血液課長
血液対策課長

(事務局席)

傍聴席

1. 大平 勝美 (おおひら かつみ)
はばたき福祉事業団理事長
2. 岡田 義昭 (おかだ よしあき)
国立感染症研究所血液・安全性研究部第一室長
3. 佐川 公矯 (さがわ きみたか)
久留米大学医学部付属病院臨床検査部教授
4. 高橋 孝喜 (たかはし こうき)
日本輸血・細胞治療学会副理事長
(東京大学医学部附属病院輸血部教授)
5. 花井 十伍 (はない じゅうご)
ネットワーク医療と人権理事
6. 山口 照英 (やまぐち てるひで)
国立医薬品食品衛生研究所生物薬品部長

(50音順、敬称略)

平成20年度第4回血液事業部会運営委員会議事要旨

日時：平成21年2月10日(火) 15:00～17:20

場所：財団法人日本教育会館(9F)喜山倶楽部「平安の間」

出席者：高松委員長、大平、岡田、高橋、花井、山口各委員

(事務局)

新村血液対策課長、林血液対策企画官、齋藤課長補佐、秋野課長補佐他
(採血事業者)

日本赤十字社血液事業本部 田所経営会議委員、日野副本部長、中島東京都センター長、碓井参事

- 議 題： 1. 議事要旨の確認
 2. 感染症定期報告について
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(審議概要)

議題1について

議事要旨に関する意見等については、事務局まで連絡することとされた。

議題2について

感染症定期報告について、事務局から説明後、質疑応答がなされた。

議題3について

事務局及び日赤から、献血者からの遡及調査の進捗状況、血液製剤に関する報告事項、献血件数及びHIV抗体・核酸増幅検査陽性件数について説明後、下記のような意見が出された。

- 地域の献血の HIV 抗体陽性率については、検査施設のキャパを大きくする等、全体の補足率を上げる努力をしていただきたい。
- いろいろな関係医療機関の努力で献血者が増えたことは大変よいことだと思うが、HIV の献血者の割合が高いことが献血推進の足を引っ張っているのは残念である。HIV 抗体陽性者の献血の割合を下げていくような対策をお願いしたい。

議題4について

日赤から、車検満了日を経過した移動採血車を運行した事例、献血者健康被害救済制度の運用状況、問診票改定、CLEIA 法の評価について報告後、下記のような意見が出された。

- 不測の事態が起きないように、採血車だけでなく、血液事業に関わる採血の道具、検査の機械も含めて、個人に依存しない形等、しっかりと管理するシステムの整備をお願いしたい。
- VVR は20～30分以内に3分の2が起こっているが、時間が経過しても起こっており、その方たちについても対策をするべきである。

議題5について

事務局から、議題その他として血液凝固第Ⅷ因子製剤及び第Ⅸ因子製剤におけるインヒビターに係る対応、注射用ノボセブン1.2mg及び同4.8mgの不採算品再算定、フィブリノゲン製剤及び血液凝固因子製剤に関する公表等について報告後、下記のような意見が出された。

- ノボセブンの不採算品再算定のような事例について、今後も運営委員会で需給の問題として取り上げていただきたい。

また、輸血療法の実施に関する指針の一部改正案及び血液製剤の使用指針の一部改正案について委員より了承された。

以上

感染症定期報告に関する今後の対応について

平成16年度第5回
運営委員会確認事項
(平成16年9月17日)

1 基本的な方針

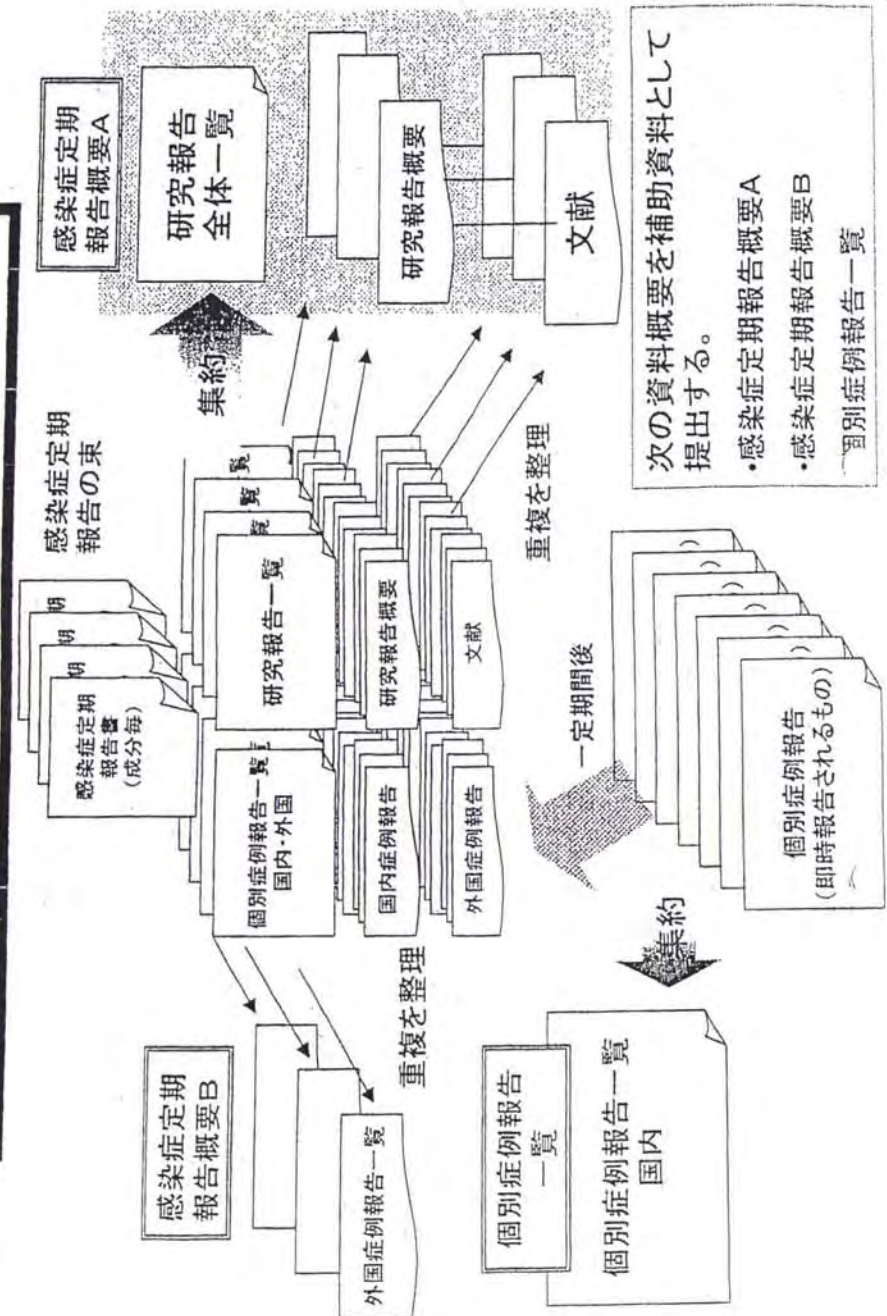
運営委員会に報告する資料においては、

- (1) 文献報告は、同一報告に由来するものの重複を廃した一覧表を作成すること。
- (2) 8月の運営委員会において、国内の輸血及び血漿分画製剤の使用した個別症例の感染症発生報告は、定期的にまとめた「感染症報告事例のまとめ」を運営委員会に提出する取り扱いとされた。これにより、感染症定期報告に添付される過去の感染症発生症例報告よりも、直近の「感染症報告事例のまとめ」を主として利用することとする。

2 具体的な方法

- (1) 感染症定期報告の内容は、原則、すべて運営委員会委員に送付することとするが、次の資料概要を作成し、委員の資料の確認を効率的かつ効果的に行うことができるようにする。
 - ① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式(別紙様式第2)及び該当文献を添付した「資料概要A」を事務局が作成し、送付する。
 - ② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧(別紙様式第4)をまとめた「資料概要B」を事務局が作成し、送付する。
 - ③ 感染症発生症例報告のうち、発現国が「国内」の輸血による症例及び血漿分画製剤の使用による感染症症例については、「感染症報告事例のまとめ」を提出することから、当該症例にかかる「資料概要」は作成しないこととする。ただし、運営委員会委員から特段の議論が必要との指摘がなされたものについては、別途事務局が資料を作成する。
- (2) 発現国が「外国」の感染症発生症例報告については、国内で使用しているロットと関係がないもの、使用時期が相当程度古いもの、因果関係についての詳細情報の入手が困難であるものが多く、必ずしも緊急性が高くないと考えられるものも少なくない。また、国内症例に比べて個別症例を分析・評価することが難しいものが多いため、緊急性があると考えられるものを除き、その安全対策への利用については、引き続き、検討を行う。
- (3) 資料概要A及びBについては、平成16年9月の運営委員会から試験的に作成し、以後「感染症の報告について(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



感染症定期報告概要

(平成21年5月14日)

平成20年12月1日受理分以降

- A 研究報告概要
- B 個別症例報告概要

A 研究報告概要

- 一覧表（感染症種類毎）
- 感染症毎の主要研究報告概要
- 研究報告写

研究報告のまとめ方について

1 平成20年12月1日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

2 一覧表においては、前回の運営委員会において報告したもの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(2008/12/1~2009/2/28)

血対D	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90064	2008/12/01	80762	B型肝炎	Clin Infect Dis 2008; 47: e52-56	2000年1月から2004年12月に日本で新たにB型肝炎表面抗原陽性となった患者を調査したところ、552名中23名(4%)がHBV再活性化で、529名が急性B型肝炎であった。再活性化群は急性B型肝炎群に比べ、年齢およびHBV DNA値が有意に高く、ALTおよびアルブミン値は低かった。また再活性化群の4分の1の患者が劇症肝不全となり、死亡した。肝臓関連死亡率は再活性化群の方が有意に高かった。	
90064	2008/12/01	80762	B型肝炎	FDA/GBR 2008年5月 業界向けガイダンス(案)	FDAはB型肝炎コア抗原に対する抗体(抗HBc抗体)が陽性となったために供血延期となった供血者のリエン・トリ・アルゴリズムを提案するガイダンス案を発表した。これまで、抗HBc抗体が2回以上陽性となった供血者は無期限に供血延期とされていたが、本ガイダンスでは2回目に陽性となった後、8週間以上経ってからHBs抗原、抗HBc抗体および高感度HBV NATによってHBV感染が否定された場合は供血可能となる。	
90078	2009/01/26	80844	B型肝炎	J Hepatol 2008; 48: 1022-1025	スロヴェニアで、HBs抗原陽性で抗HBc抗体陽性、抗HBs抗体低力陽性、HBV DNA陽性の濃厚赤血球と新鮮凍結血漿を輸血された59歳の患者が4ヶ月後に急性B型肝炎を発症した。また同じ供血血液由来のRCCの輸血を受けた71歳の患者も7ヶ月後にHBV感染を認めた。2例ともドナーと同じ配列を有するジェノタイプDが感染していた。潜在的B型肝炎ウイルス感染者の血液は抗HBs抗体が陽性にもかかわらず、感染性を有した。	
90068	2008/12/17	80784	B型肝炎	J Med Virol 2008; 80: 1880-1884	1971~2005年の35年間に虎ノ門病院に来院した急性HBV感染患者153名および慢性HBV感染患者4277名について5年間毎のHBVジェノタイプ/サブジェノタイプを調べた。急性感染患者数は35年間で増加し続けた。慢性感染患者は1986~1990年が最大であった。ジェノタイプは急性感染患者と慢性感染患者で大きく異なった(A、B、C型: 28.6%、10.3%、59.5% vs 3.0%、12.3%、84.5%)。最近では外国のサブジェノタイプB2/Baが増加する傾向がある。	
90078	2009/01/26	80844	B型肝炎	Transfusion 2008; 48: 1602-1608	供血時には血清検査陰性であったが、その後HBV DNAが検出された供血者由来の血液成分を輸血された2名の免疫不全患者について調べた。受血者1はHBVワクチン接種を受け、抗HBsキャリアであったが、赤血球輸血後13ヶ月で急性B型肝炎を発症するまで他のHBVマーカーは全て陰性であった。供血者とHBVシークエンスが一致したため、輸血関連感染と確認された。受血者2は血小板輸血を受けたが、感染していなかった。	1
90064	2008/12/01	80762	B型肝炎	Transfusion 2008; 48: 286-294	最小感染量を求めるために、遺伝子型Aまたは遺伝子型CのHBVを含む急性期前の接種株をチンパンジーに接種したところ、最小50%チンパンジー感染量(CID50)は各々約10コピーと推定された。最低感染量を接種したチンパンジーにおけるHBV DNA ウィンドウ期は遺伝子型Aでは55-76日、遺伝子型Cでは35-50日。HBs Ag ウィンドウ期は遺伝子型Aでは69-97日、遺伝子型Cでは50-64日であった。またHBV DNAダブリングタイムは遺伝子型Cの方が遺伝子型Aに比べ有意に短かった。	
90068	2008/12/17	80784	B型肝炎	Vox Sanguinis 2008; 95: 174-180	HBV DNA陽性かつ表面抗原(HBsAg)陽性オカルトHBV感染の検出感度を上げるために、HBV DNAとHBsAgを同時に濃縮する新規方法を開発した。二価金属存在下でpoly-L-lysineでコートした磁気ビーズを使用し、ウイルス凝集反応を増強させ、ウイルスを濃縮する方法により、HBV DNAとHBsAg量は、最高4~7倍に濃縮された。本方法により、EIAとHBV NATの感度が上昇し、HBsAg EIAを用いてオカルトHBV感染者40名のうち27名を検出することができた。	2

血対D	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90064	2008/12/01	80762	B型肝炎C型肝炎	第58回日本輸血・細胞治療学会総会 2008年4月25-27日 P-033	2007年に医療機関から日本赤十字社に報告された輸血関連感染症の報告数は124例(10月末現在)であり、一昨年及び昨年の同期間に比べ減少傾向にある。内訳はHBVが61例、HCV32例、細菌24例、その他のウイルスが7例であった。ウイルス感染(疑)症例の調査結果により病原体を確認した症例は、HBVの12例とHCVの1例であった。HCVの1例は20プールNAT開始後(2004年8月開始)初めての検出限界以下の献血血液による感染症例であった。	
90072	2008/12/17	80788	C型肝炎	第70回日本血液学会総会 2008年10月10-12日	症例は再生不良性貧血の54歳の女性で、2007年6月26日に初回輸血が実施され、初回輸血前検査はHCV抗体陰性、HCVコア蛋白陰性で、あった。10月1日の輸血後、HCVコア蛋白が陽性化したため、再検査を開始した。患者には計54本の輸血があり、保管検体の個別NATにより、1検体からHCV-RNAを検出した。患者と献血者のHCV Core-E1-E2領域の塩基配列が一致したことから、本症例は輸血によるHCV感染である可能性が極めて高い。	3
90064	2008/12/01	80762	C型肝炎	Clin Infect Dis 2008; 47: 627-633	フランスの大学病院の血液透析ユニットでのHCV伝播リスクにおける環境汚染および標準的注意の非遵守の役割を評価した。試験期間中にHCV陽性となった2名のうち1名は、同ユニットで治療中の慢性感染患者と同じウイルス株に感染していることが系統遺伝学的解析により明らかとなった。環境表面検体740例中82例がヘモグロビンを含み、その内6例がHCV RNAを含んでいた。手の衛生に関する遵守率は37%、患者ケアの直後に手袋をはずしていたのは33%であった。	
90064	2008/12/01	80762	C型肝炎	Clin Infect Dis 2008; 47: 931-934	ニューヨーク市のEast Harlemのクリニックから18歳以上で血中HCV PCR陽性の吸引用麻薬常習者38名の鼻汁検体および吸引に使用したストローを入手し、血液およびHCV RNAの存在の有無を調べた。鼻汁検体28例(74%)、ストロー3例(8%)から血液が検出され、鼻汁検体5例(13%)、ストロー2例(5%)でHCV RNAが検出された。HCVウイルスの鼻腔内伝播のウイルス学的妥当性が示された。	4
90064	2008/12/01	80762	E型肝炎	Am J Trop Med Hyg 2008; 78: 1012-1015	スペインでブタに感染しているヒト101名と感染していないヒト97名におけるHEV感染の有無を調べた。抗HEV IgG保有率は陽性群では18.8%、非陽性群では4.1%であった。ブタに接するヒトの抗HEV IgG保有リスクは5.4倍(P=0.03)であった。HEV感染は養豚作業員の職業病として扱うべきである。	
90078	2009/01/26	80844	E型肝炎	Transfusion 2008; 48: 1368-1375	2004年9月20日に39歳日本人男性から献血された血液はALT高値のため不適当とされ、HEV陽性であった。当該ドナーの再検査の結果、9月6日に献血を行い、HEV RNAを含有する血小板が輸血されていた。当該ドナーと親戚は8月14日にブタの肉を食べており、父親は9月14日に急性肝炎を発症し、E型劇症肝炎で死亡した。他に7名がHEV陽性であった。レシビエントは輸血22日目にALTが上昇し、HEVが検出された。	
90075	2009/01/09	80834	E型肝炎	Vox Sanguinis 2009; 95(Suppl.1): 282-283	2005年の中国の4都市(Beijing, Urmuch, KunmingおよびGuangzhou)における献血検体のHEV感染率を調べた。その結果、ルーチン検査(抗HCV、抗HIV1/2、HBsAg、梅毒およびALT)陽性献血者の約1%は抗HEV IgMまたは抗HEV Ag陽性で、HEV感染の可能性があった。また、ALTスクリーニングは中国のHEV感染血排除に役立つ可能性があった。	

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90078	2009/01/26	80844	E型肝炎	Vox Sanguinis 2008; 95: 94- 100	日本のブタから分離されたHEVジェノタイプ3または4の4株について熱処理およびフィルターによる除去の程度を検討した。HEVはアルブミン溶液中で60°C5時間加熱後およびフィブリノゲン中で60°C72時間加熱後も感染力が検出されたが、PBS中で60°C5時間加熱後およびフィブリノゲン中で80°C24時間加熱後には検出限界以下に不活化された。また、20nmナノフィルター使用により完全に除去された。	5
90084	2008/12/01	80762	E型肝炎	第56回日本輸 血・細胞治療学 会総会 2008 年4月25-27日 O-026	北海道地区において現行プールNATスクリーニングの残量を用いてTaqMan RT-PCR法によるHEV NATスクリーニングを行った。陽性献血者85例について追跡調査および過剰調査などを行なった。陽性献血者の多くは動物内臓肉を食してHEVに感染したと考えられる新規感染者で、GenotypeはG3が多かった。多くは症状が現れないまま抗体が陽転化し、典型的な無症候性一過性感染の経過をたどった。	
90084	2008/12/01	80762	HIV	ABC Newsletter 2008; No.26 2008年7月4日	米国医師会(AMA)は、男性間性行為を行った男性(MSM)の供血延期期間を生産としている連邦の方針を5年間に変更することを支持するという声明を採択した。AMAはこの新方針をFDAに通告し、この方針を推進するグループと協力していく。FDAは1977年以降、MSMの供血を生産延期することを血液事業者に要求しているが、アメリカ血液センターなどからは反対意見が出されている。	
90068	2008/12/17	80784	アメリカ・トリパノソマ症	Transfusion 2008; 48: 1862-1868	スペイン、カタルーニャ血液銀行は、高リスク献血者におけるシャーガス病スクリーニング計画を実行し、献血者集団でTrypanosoma cruzi(T. cruzi)感染の血清学的陽性率を調査した。その結果、全体の陽性率は0.62%(1770名中11名)で、最も陽性率が高かったのはボリビア人であった(10.2%)。陽性者11名中1名は、シャーガス病流行地域に数年間滞在したことのあるスペイン人であった。非流行国の高リスク献血者にT. cruziスクリーニング検査を実施する必要がある。	6
90084	2008/12/01	80762	アメリカ・トリパノソマ症	Vox Sanguinis 2008; 95(Suppl.1): 39	米国で全献血者を対象にしたTrypanosoma Cruzi検査が導入された2007年1月30日以降、最初の10ヶ月間、献血者の調査を行った。適合献血のうちELISA法で反復陽性(RR)となったのは0.013%(90/651471)で、そのうちRIPA陽性は34%(28/82)で、陽性確認率は0.0043%であった。全献血のスクリーニングは費用対効果が低く、出生地と初回献血者に絞った対策の検討が示唆された。	
90064	2008/12/01	80762	インフルエンザ	Vox Sanguinis 2008; 95(Suppl. 1): 40	米国におけるパンデミックインフルエンザの血液供給に対する影響をシミュレーションした。3ヶ月間の血液供給量が50%減少した場合、血液需要に制限がない場合は在庫のほとんどを使い尽くしたが、血液の使用を必要最低限に制限した場合は在庫がなくなることはなかった。	
90068	2008/12/17	80784	ウイルス感染	BuaNews online 2008年 10月13日	南アフリカ、ヨハネスブルグで3名の死者を出したウイルスは、暫定的に西アフリカのラッサウイルスに近い、齧歯類媒介性アレンウイルスであると特定された。国立感染症研究所と保健省は共同で、このウイルスが体液を介してヒトからヒトに感染するため、「患者の看護に特別な予防的措置が必要である」との声明を発表した。3名の死因を確定するには更なる検査が必要である。	7

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90064	2008/12/01	80762	ウイルス感染	PLoS Pathogens 2008; 4: e1000047	出血熱症例の小さな流行が、2003年12月と2004年1月にボリビアのCochabamba付近で発生した。1死亡例から検体入手し、患者血清検体から非細胞障害性ウイルスを単離し、アレンウイルスと同定した。RT-PCR分析、並びにS及びL RNAセグメント配列の解析の結果、このウイルスはサブアウイルスに最も近縁であるが、新規のウイルスであることが示された。我々はこのウイルスをChapareウイルスと命名することを提案する。	
90066	2008/12/16	80781	ウイルス感染	Proc Natl Acad Sci USA 2008; 105: 14124- 14129	インフルエンザ様疾患の小児の呼吸分泌物中から、汎ウイルスマイクロアレイ法を用いて、初めてヒトカルディオウイルスを同定した。系統遺伝学的分析から、このウイルスはTheilerのネズミ脳脊髄炎ウイルス型に属し、Saffoldウイルスと最も近縁であった。また、胃腸疾患患者群498名から得た751例の糞便検体中6検体からカルディオウイルスが検出された。	
90064	2008/12/01	80762	ウイルス感染	ProMED- mail20080720.2 201	オーストラリアBrisbaneの動物病院のスタッフが致死性のヘントラウイルスに感染した。看護師1名と獣医1名が、感染したウマ数頭を治療後、感染した。前回のアウトブレイクは1994年で副教師1名とウマ14頭が死亡した。同ウイルスがヒト-ヒト感染するとのエビデンスはなく、拡大する危険性はない。	
90066	2008/12/16	80781	ウイルス感染	ProMED- mail20081028.3 409	2008年10月初旬に南アフリカでアレンウイルスによる感染のアウトブレイクが同定された。9月12日から10月24日までに計5例が報告され、5例中4例が死亡し、1例は入院中である。死亡した4例では発病から死亡まで9-12日間であった。塩基配列分析より、ユニークな旧世界アレンウイルスが原因であることが明らかとなった。現在のところ新たな疑い症例はない。	8
90075	2009/01/09	80834	ウイルス感染	Transfusion 2008; 48: 1180-1187	米国テキサス南東部の健康な成人ドナー100名の血液中のヒトヘルペスウイルス(HHV)陽性率とウイルスDNA量をRT-PCRにより調べた。その結果、HSV-1、HSV-2、VZV及びHHV-8 DNAはどの検体からも検出されなかった。一方、EBVは72%、HHV-7は65%、HHV-6は30%、CMVは1%に検出された。また、1名の血液から6.1 × 10 ⁷ geq/mlを超えるHHV-6 Type Bが検出されたが、健康者における異常な高値は活動性感染や免疫不全とは関連が無いと思われる。	
90075	2009/01/09	80834	ウイルス感染	WHO/EPR 2008年10月13 日	南アフリカとザンビア出身者の最近の死亡例3例はアレンウイルス科のウイルスが原因であることが、NICDおよびCDCで行われた検査の結果明らかとなった。このウイルスに関する詳細な分析が継続されている。一方、南アフリカでは患者と密接に接触した看護師が感染し、入院中である。	
90066	2008/12/16	80781	ウイルス性 脳炎	ProMED- mail20080828.2 697	インド東部のウッタルプラデシュ州で小児が死亡させている原因不明のウイルスは、インド保健省の専門家により急性脳炎症候群と診断された。同州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。血液検査で日本脳炎陽性となった患者は5%以下であった。日本脳炎とエンテロウイルスとの混合感染の可能性について調査中である。	
90068	2008/12/17	80784	ウエストナ イルウイルス	ABC Newsletter No.38 2008年 10月17日	2008年9月に、イタリアで何年かぶりにヒトのウエストナイルウイルス(WNV)脳炎が2例報告された。1例目はFerraraとBolognaの間に住む80歳の女性、2例目はFerraraに住む60代後半の男性であった。また、ウマ6頭とトリ1羽でWNV感染が確認された。WNV髄膜炎の積極的サーベイランスプログラムが開始され、当該地域で献血者スクリーニング用NATが導入された。また、当該地域に1日以上滞在したことのある献血者を28日間供血延期する措置がとられた。	9

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90064	2008/12/01	80762	ウエストナ イルウイルス	Rev Panam Salud Publica 2008; 19: 112- 117	文献および未発表データから、ラテンアメリカやカリブ海地域のウ エストナイルウイルス(WNV)感染の現状をまとめた。WNV感染は 2001年にCayman諸島とFlorida Keysの住民で見られ、2002～ 2004年にジャマイカ、メキシコなど周辺地域で動物や鳥類での感 染が確認されている。しかし、疾患報告数は少ない。この不可解 な熱帯生態系でのウイルス減弱または他の可能性を検討するた めには分離株が必要である。	
90068	2008/12/17	80784	クロイツフェ ルト・ヤコブ 病	J Neurol Neurosurg Psychiatry 2008; 79: 229- 231	オーストリアの39歳男性が感覚異常などの神経症状で入院後、急 速に悪化し、4ヶ月後に死亡した。組織学的検査で海綿状変化、神 経細胞脱落及びグリオシスが、免疫組織化学的検査でびまん 性シナプティックな異常プリオンの沈着が見られ、CJDと診断され た。また患者のPRNPは129Met-Metであった。患者は22年前まで 死体由来のヒト成長ホルモン(hGH)製剤治療を受けており、医原 性リスクが認められるため、孤発性若年性CJDの可能性も否定で きないが、WHO基準により確定医原性CJDと分類された。	10
90064	2008/12/01	80762	コンゴ・クリ ミア出血熱	ProMED- mail20080709.2 092	2008年7月7日、トルコのBursa, CanakkaleおよびSamsunの病院で ダニ媒介性疾患であるクリミア・コンゴ出血熱により3名が死亡し、 この2ヶ月での死者数は37名となった。保健省はダニに注意するよ う呼びかけ、咬まれた場合は決して手でつぶさず、医師にピン セットで注意深く取り除いてもらい、コードで消毒することを推奨し ている。	
90064	2008/12/01	80762	サルモネラ	CDG 2008年7 月8日	CCDCは関係機関と協力して複数の州で発生したサルモネラ血清 型セントポールアウトブレイクを調査している。生のトマトの摂取 が原因と考えられている。2008年4月以降2008年7月7日までに、 米国の41の州、ワシントンD.C.およびカナダで991名の患者が同じ 遺伝子パターンでサルモネラ血清型セントポールに感染したことが 確認された。	
90075	2009/01/09	80834	チクングニ ヤウイルス 感染	Transfusion 2008; 48: 1333-1341	2005年から2007年に、チクングニヤウイルス(CHIKV)はレユニ オン島で大流行し、供血は2006年1月に中断された。大流行中のウ イルス血症血供の平均リスクは、10万供血あたり132と推定され た。2006年2月の最流行時におけるリスクは、10万供血あたり 1500と最高であった。この期間中、757000人の住民のうち推定 312500人が感染した。2006年1月から5月の平均推定リスク(0.7%) は、CHIKV NAT検査による血小板出血のリスク(0.4%)と同じオー ダーであった。	
90064	2008/12/01	80762	デング熱	Hong Kong Med J 2008; 14: 170-177	1998～2005年に香港の公立病院に入院したデング確定患者全員 の医療記録をレトロスペクティブに検討した。126例中123例(98%) がデング熱、3例(2%)がデング出血熱であった。1例が輸血により 感染したデング熱であった。116例が輸入症例、10例が地域症例 であった。デングウイルス1型が最も多く、次に2型、3型、4型の順 であった。死亡例はなかった。発熱、皮疹を呈し、血小板減少など を示す歴のある患者には鑑別診断にデング熱を含めるべき である。	
90075	2009/01/09	80834	デング熱	Transfusion 2008; 48: 1342-1347	高力価の培養デングウイルス セロタイプ2をアルブミンおよび免 疫グロブリンの各種製造工程(低温エタノール分画、陽イオン交換 クロマトグラフィー、低温殺菌、S/D処理およびウイルスろ過)前 の検体に加え、各工程での同ウイルスのクリアランスをVero E6細胞 培養におけるTCID50アッセイおよびRT-PCRで測定した。その結 果、全ての工程が不活化・除去に有効であることが示された。	
90075	2009/01/09	80834	デング熱	Transfusion 2008; 48: 1348-1354	2005年9月20日～12月4日のプエルトリコの米軍赤十字における すべての供血16521検体中のデングウイルス(DENV) RNAを TMA(transcription-mediated amplification)法で測定したところ、12 検体(0.07%)がTMA陽性であった。4検体は、RT-PCR(DENVセロ タイプ2および3)陰性であった。RT-PCR陽性4検体中3検体でウ イルスを培養することができた。TMA陽性12検体中1検体がIgM陽性 であった。1:16に希釈した場合は5検体のみTMA陽性であった。	

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90064	2008/12/01	80762	バベシア症	American Society for Microbiology 108th General Meeting 2008 年6月1-5日、 Boston	米国中南部では稀な輸血によると考えられるBabesia microti感染 症例の報告である。61歳の女性患者で、赤血球輸血後、吐き気と 発熱を訴え、敗血症の症状を呈し、死亡した。血液塗抹標本で赤 血球の5～15%にトロフォゾイト(栄養体)があった。患者血液検体 中でBabesiaは形態学的に確認され、PCRでB. microti陽性であっ た。輸血された製剤の供血者のうち1名がB. microti陽性であっ た。	
90075	2009/01/09	80834	バルボウ ウイルス	FDA/CBER 2008年7月 案 件向けガイダ ンス(案)	血液由来製品によるバルボウウイルスB19伝播リスクを低減するた めの核酸増幅検査(NAT)についてのガイダンス案が示された。全 ての血液由来製剤について、製造プール中のバルボウウイルスB19 DNAのウイルス負荷を確実に10000 IU/ml未満とするため、製造 過程の品質管理検査としてNATを実施すべきである。ミニプール 中でのNATの感度は少なくとも1000000 IU/mlとするべきである。 これらの基準を超えるものは使用してはならない。	
90076	2009/01/26	80844	バルボウ ウイルス	Lab Hematol 2007; 13: 34- 38	血液交換、コレチコステロイドおよびコリンエステラーゼ阻害剤に よる治療を受けていた重症筋無力症患者が、アルブミンを用いた 血液交換を行った2週後にバルボウウイルスB19感染による赤芽球 減少症と診断された。アルブミン由来感染かどうかを確定するこ とはできなかったが、アルブミンなどの血液製剤によるB19感染を除 外することはできない。	11
90064	2008/12/01	80762	バルボウ ウイルス	Transfusion 2008; 48: 1036-1037	大阪における1997～1999年の献血者979052名中102名がヒトバル ボウウイルスB19感染者であった。B19感染者のうち20名のB19 DNA、IgGおよびIgMを長期間フォローアップしたところ、B19持続感 染が観察されたが、B19感染の症状を報告した者はいなかった。 B19急性感染後の血液ウイルス価は約1年で10 ⁴ IU/mL未満、約 2年で10 ³ IU/mL未満まで下がることが示された。	
90064	2008/12/01	80762	ハンタウ ウイルス	Emerg Infect Dis 2008; 14: 808-810	スウェーデンにおけるPuumaalaウイルスの予期せぬ大規模アウト ブレイクにより、2007年のVästerbotten地方の流行性腎症患者の 数は100,000人当たり313人に至った。腎臓病の増加の他、気候温暖 化および地表を覆う積雪の減少により、ウイルスを媒介するハタ ネズミの活動が活発だったことが、当該アウトブレイクの一因であ ろうと考えられる。	
90064	2008/12/01	80762	ブルセラ症	Clin Infect Dis 2008; 46: e131-136	急性ブルセラ症患者39名の血液検体中のBrucella DNAの存在を RT-PCR法により調べた。その結果、治療終了時では87%、治療 完了後6ヶ月では77%、治療後2年を過ぎても70%の患者で、無症候性 であるにもかかわらず、Brucella DNAが検出された。適切な治療 を行い、回復したように見えても、Brucella DNAは持続する。ブル セラ菌は除去不可能な持続性の病原体である。	
90064	2008/12/01	80762	マラリア	Emerg Infect Dis 2008; 14: 1434-1436	2007年にマレー半島でフィランランドの旅行者が、通常はマラリ アの原因となる二日熱マラリア原虫に感染した。二日熱 マラリア原虫はヒトマラリアを引き起こす第5のマラリア原虫種と して確立された。この疾病は生命を脅かす危険があり、臨床医と臨 床検査技師は旅行者においてこの病原体を更に注意すべきであ る。	12
90064	2008/12/01	80762	リケッチ ア症	Emerg Infect Dis 2008; 14: 1019-1023	ネコ/ミが媒介するRickettsia felis感染症のヒト症例は世界中で報 告されている。症状は発疹熱やデング熱などに類似しており、実 際よりも少なく推定されている可能性が高い。ヒトの健康を脅かす 感染症として今後調査が必要である。	
90066	2008/12/16	80781	リケッチ ア症	ProMED- mail20080728.2 306	オランダ・ブラバント州の公衆衛生局が行った調査でQ熱の症例 報告数が急激に増加し、2008年7月21日付けで491症例が報告さ れている。感染症管理センター長によると、実際の感染者数は報 告された症例数の10倍であると思われる。2007年まではQ熱はオ ランダではほとんど存在しなかった。	

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90066	2008/12/16	80781	レプトスピ 症	Infect Genet. Evol 2008; 8: 529-533	コスタリカにおいて、レプトスピラ症の入院患者から分離されたレ プトスピラは、Javanica血清型に分類される新しい血清型で、 Arenalと命名された。同じ地区の重症患者から分離された株も同 じ血清型であったことから、この株は、この地域に流行する新規の 高病原性の血清型であると考えられた。	
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	2008年プリオン 研究会 2008 年8月29-30日	CJDサーベイランス委員会による調査では1999年4月から2008年 2月までの9年間に日本国内で1089例がプリオン病と判定された。 うち孤発性CJDが821例(76.8%)、遺伝性プリオン病が171例 (16.0%)、硬膜移植後CJD74例(6.8%)、変異型CJD1例(0.1%)、分類 不能2例(0.2%)であった。日本のプリオン病剖検率は欧米諸国より 著明に低かった。孤発性CJDの病型は欧米に比べMM2型が多 かったが、非典型例が多く剖検されている可能性が考えられた。	13
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	2008年プリオン 研究会 2008 年8月29-30日 ポスター11	ウイルス除去膜濾過工程を含んでいる製剤(血液凝固第VIII因子 製剤: プラノバ20N濾過、抗HBe免疫グロブリン製剤: プラノバ 35N濾過)について、263K株感染ハムスターより得たSUS処理 PrPScを用いて、その除去効果を検証した。その結果、SUS処理 PrPScは濾過膜の孔径よりも小さいにもかかわらず、プラノバ35N やプラノバ20Nで除去された。PrPScが凝集したり、膜へ吸着した ためと考えられる。	14
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	2008年プリオン 研究会 2008 年8月29-30日 ポスター18	スクレイビー263K感染ハムスター脳乳剤を脳内接種したハムス ターにおける血中PrPres経時の変化を追跡したところ、PK抵抗性 3F4反応性蛋白バンドは、感染後4~6週で認められ、10週ではほ ぼ消失した。発症末期では血中PrPresと見られる蛋白バンドは認 められなかった。PrPresをマーカーとした血液検査は感染後発症 前〜発症中期までに限定される可能性が示唆された。	15
90075	2009/01/09	80834	異型クロイ ツフェルト・ ヤコブ病	American Society of Hematology/Pr ess Releases 2008年8月28日	Blood誌のprepublished onlineに掲載されたヒツジにおける研究に よると、輸血によるBSE伝播のリスクは驚くほど高い。エジンバラ 大学で行われた9年間の研究は、BSEまたはスクレイビーに感染し たヒツジからの輸血による疾病伝播率を比較した。その結果、BSE およびスクレイビーとも輸血によりヒツジに効率よく伝播された。症 状を呈する前のドナーから採取された血液によっても伝播すること が示された。	
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	Ann Neurol 2008; 53: 697- 708	米国の国立プリオン病病因調査センターの患者11名(平均発症年 齢62歳)を調べたところ、海綿状変性の型、PrP免疫染色パターン およびマイクロプラークの存在が、既知のプリオン病とは異なり、 通常の方法では典型的なプロテアーゼ感受性PrPは検出されな かった。我々はこれらをプロテアーゼ感受性PrP(PSPr)と 名付けた。PSPrは、プリオン病の中では稀ではなく、我々のデータ が示すよりもさらに多い可能性がある。	
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	Blood, Prepublished online 2008年7 月22日	ヒツジを用いた感染実験において、BSEは36%、スクレイビーは43% と予想以上に高い輸血伝播率を示した。高い伝播率および臨床 的に隠性のレシビエントにおける比較的短期間の一定した潜伏期 間は、血中の感染性力価が高いことおよびTSEが輸血により効率 的に伝播することを示唆する。血液製剤によるヒトでのvCJD伝播 を研究するために、ヒツジが有用なモデルであることが示された。	16

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90075	2009/01/09	80834	異型クロイ ツフェルト・ ヤコブ病	Cell 2008; 134: 757-768	マウスPrPScと混合させることによって折り畳み異常が起こったハ ムスターPrP ^{Sc} は、野生型ハムスターに対して感染性を起こす新規 なプリオンを生成した。同様の結果は、反対方向でも得られた。 PMCA増幅を繰り返すとin vitro産生プリオンの順応が起こる。この プロセスは、in vivoでの連続世代に観察される株の安定性を暗示 させる。種の壁と株の生成がPrP折り畳み異常の伝播によって決 定されることが示唆される。	
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	Emerg Infect Dis 2008; 14: 1406-1412	263Kスクレイビーの臨床症状を呈するハムスター22匹の尿にTSE 感染性があることが示された。これらの動物の腎臓と膀胱のホモ ジネートは20000倍以上希釈してもTSE感染性があった。組織学 的、免疫組織化学的分析では、腎臓における疾患関連PrPの散発 的な沈着以外、炎症や病変は見られなかった。尿中のTSE感染性 が、自然のTSEの水平感染に何らかの役割を果たす可能性がある 。	
90064	2008/12/01	80762	異型クロイ ツフェルト・ ヤコブ病	PLoS ONE 2008; 3: e2878	野生型マウスおよびヒトPrPを発現しているトランスジェニックマウ スに、輸血関連vCJD感染第1号症例由来の脳材料を接種し、輸 血によるヒト-ヒト間の2次感染後のvCJD病原体の性質について 調べた。その結果、潜伏期間、臨床症状、神経病理学的特徴およ びPrP型について、vCJD(輸血)接種群はvCJD(BSE)接種群と類 似していた。vCJD病原体は、ヒトにおける2次感染により、有意な 変化が起こらないことが明らかとなった。	
90068	2008/12/17	80784	異型クロイ ツフェルト・ ヤコブ病	PLoS ONE 2008; 3: e3017	非定型BSE(BASE)に感染した無症候のイタリアの乳牛の脳脊液モ ニターをカニクイザルに脳内接種した。BASE接種サルは生存期間 が短く、古典的BSEまたはvCJD接種サルとは異なる臨床的展開、 組織変化、PrPresパターンを示した。感染牛と同じ国の孤発性 CJD患者でPrPが異常なウエスタンブロットを示す4例のうち3例の PrPresに同じ生化学的特徴を認めた。BASEの置長類における高 い病原性および見かけ上孤発性CJDである症例との関連の可能 性が示唆された。	
90064	2008/12/01	80762	感染	Transfusion 2008; 48: 304- 313	血小板濃厚液におけるUVC照射の病原体不活化能を検討した。 UVC照射は、血小板の品質に影響を及ぼさず、細菌(表皮ブ ドウ球菌、黄色ブドウ球菌および大腸菌)ならびに伝播性胃腸炎ウ イルスなど広範囲ウイルス(HIVおよびシミアンウイルス40を除く) を不活化することができた。しかし、HIVのような血液感染性ウイル スに対応するには、UVC法をさらに最適化することが必要である。	
90064	2008/12/01	80762	感染	Transfusion 2008; 48: 697- 705	欧州の3つの血液センターにおけるアモトザレンおよびUVAによる フォトケミカル処理(PCT)過程のプロセスバリデーション試験を行っ た。フィブリノーゲンおよび第VIII因子はPCTにより平均26%減少し たが、治療用血漿として十分なレベルを保持していた。他の凝固 因子は対照FFPのレベルの81-97%であった。PCT処理済FFP中の 凝固因子が治療用血漿に関する欧州規制および国内基準の範 囲内に保持されることが示された。	
90064	2008/12/01	80762	感染	Vox Sanguinis 2008; 94: 315- 323	アモトザレンと紫外線A波で光化学処理した血小板(PCT-PLT)の 輸血に関連する有害事象を顕現するために能動的血液安全監視プ ログラムを実施した。患者1400名に7437件のPCT-PLTが輸血さ れ、その内、68件が有害事象と関連付けられた。PCT-PLT輸血に 関連した急性輸血反応は発現頻度が低く、ほとんどが軽度であ った。	

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90064	2008/12/01	80762	感染	Vox Sanguinis 2008; 95(Suppl. 1), 2A-S01-02	化学的または光化学的遺伝子修飾に基づいた血液製剤中の病原体不活化 (PI) は広範囲のスペクトルの予防的アプローチである。溶媒界面活性剤 (SD) およびメチレンブルー法は欧州の多くの国で使われている。アモトサレン (Intercept)、リボフラビンを用いた新しい方法が導入されている。リボフラビン、UV および可視光線を用いる血小板 (PC)、血漿および赤血球のための PI が開発中である。	
90075	2009/01/09	80834	狂犬病	ProMED-mail20080826.2 660	1990年から2007年の中国における狂犬病発生傾向を調べた研究によると、最近8年間でヒト狂犬病症例数が急激に増加したことが明らかとなった。ヒト狂犬病は1990年から1996年の間は全国的な狂犬病ワクチン接種プログラムにより抑制され、わずか159症例が報告されただけであるが、2006年は3279症例と激増した。	
90064	2008/12/01	80762	原虫感染	Emerg Infect Dis 2008; 14: 1013-1018	リーシュマニア症は生物媒介性疾患で、南ヨーロッパに定着しており、毎年700例近く、トルコを含めると3950例のヒトでの感染が報告されている。無症候症例は臨床症例の30〜100倍とみられ、また飼育犬の血清陽性率は25%と推定される。薬剤耐性 Leishmania infantum がイスを介して拡大するおそれもある。全ヨーロッパレベルでの研究が必要である。	
90068	2008/12/17	80784	細菌感染	Am J Infect Control 2008; 36: 602	減菌法として両耳の上部耳介軟骨に置き鍼治療 (Stapling) を受けた16歳の女性が、2週間後に左耳の鍼周囲の紅斑および圧痛を呈した。膿瘍ドレナージ後の培養および感受性試験の結果、両耳で著しい緑膿菌の生育が認められた。21日間の経口シプロフロキサシン投与により回復した。外耳軟骨は、血流に乏しく特に感染しやすい。耳鍼が危険な緑膿菌感染を起こす可能性があることを医師は認識するべきである。	17
90072	2008/12/17	80788	細菌感染	American Society for Microbiology 108th General Meeting 2008 年6月1-5日	マサチューセッツの医療センターで品質管理のために使用された廃棄製剤、使用期限切れロット、アフェレーシスの残りの人血清アルブミン製剤を入手し、クラミジアの有無を調べた。その結果、PCR 及びウエスタンブロットにより、4社の20製剤全てにおいてクラミジアの存在が確認された。また、in vitro 培養を行ったところ11株体 (55%) でクラミジア生育が生じた。	
90066	2008/12/16	80781	細菌感染	CDC/MMWR 2008; 57: 1145-1148	米国ミネソタ州の68歳男性が、2007年10月12〜21日に手術後の輸血を受け、敗血症および多臓器不全をきたした後、10月31日に発熱を伴う急性血小板減少症を発現し、11月3〜5日の血液検体からPCR 及び抗体検査でアナプラズマ症感染が確認された。血液ドナーの1人に A. phagocytophilum 陽性がPCR 及びIFA 検査で確認され、血液ドナーに感染源が確認された初の事例となった。	18
90064	2008/12/01	80762	細菌感染	Transfusion 2008; 48: 1520-1521	骨髓形成症候群と汎血球減少症の79歳男性が、血小板輸血と続いて赤血球1単位の輸血を受けた。40分後に39.6℃の発熱、硬直、背部痛、低血圧および低酸素症を呈し、輸血は中止された。患者は抗菌剤による治療で回復した。患者の血液および赤血球バッグの残存物から Streptococcus pneumoniae 血清型4が検出された。赤血球輸血による S pneumoniae 感染の初めての症例である。	
90064	2008/12/01	80762	細菌感染	第56回 日本 輸血・細胞治療 学会総会 2008年4月25- 27日 WS-3-3	血小板濃厚液の輸血後に、TRALI 様の急性呼吸不全と髄膜炎を併発し、血小板凝集液から Bacillus cereus が検出された症例の報告である。TRALI 様の急性呼吸不全を呈した際は、輸血後感染症も視野に入れた対応が必要である。髄膜炎併発例の報告はこれまでに無いが、輸血後感染症治療では髄液移行性も考慮した抗生剤選択が求められる。培養検査だけでなく、遺伝子検査まで施行することが、診断及び同一菌株の証明に重要である。	

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90077	2009/01/21	80839	鳥インフル エンザ	Proc Natl Acad Sci USA 2008; 105: 7558- 7563	ユーラシアおよび北米系統のH7型トリインフルエンザウイルスの受容体結合能およびフェレットモデルにおける感染性を調べた。その結果、2004年にカナダで分離されたH7N3型、2002-2003年に米国北東部で分離されたH7N2型はα2-6結合シアル酸に対する親和性を高めたHAを保有していた。また2003年にニューヨークの男性から分離された低病原性H7N2型はフェレットの上気道で効率的に増殖し、直接接触で感染できることが確認された。	
90064	2008/12/01	80762	鳥インフル エンザ	ProMED-mail20080825.2 548	タミフル耐性型の「通常の」季節性インフルエンザが急速に拡大しており、南アフリカでは今年の冬(2008〜2009年)のインフルエンザに効果がないおそれがある。WHO のデータによると同国で H1N1 株に感染した107名に関する検査の結果、全員がタミフルに耐性の突然変異株を保有していた。2008年4月1日から8月20日に南半球の12カ国の H1N1 インフルエンザ感染患者由来検体788例中242例(31%) がタミフル耐性に関係がある H274Y 突然変異を有していた。	19
90064	2008/12/01	80762	梅毒	SignOnSanDiego.com 2008年 3月26日	カリフォルニア州サンディエゴ郡の年間梅毒症例数は、最低となった2000年の28例から昨年(2007年)は340例まで急増した。州の他の大都市の郡と比べて非常に急激な増加である。増加率は州全体の2倍以上、全国の3倍以上になる。州から派遣された5名の専門家チームは、梅毒と診断された人々と連絡をとって、性的パートナーを探し、検査を受けるよう勧めている。	

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	赤血球、血小板	研究報告の公表状況	Transfusion (United States) Aug2008, 48 (8) p1602-8	公表国	使用上の注意記載状況・その他参考事項等
販売名(企業名)	—			米国	
供血時点にはB型肝炎に関する血清検査で陰性であったが、その後HBV DNAが検出された供血者から血液成分(赤血球、血小板)の輸注を受けた2例の免疫不全患者について報告する。 供血者は39歳男性で、供血時点は血清検査陰性であったが、6週間後に採取した検体では抗Hbc抗体陽性(HBs抗原、抗HBs抗体は未検出)となり、その後の検査でHBV DNAが検出された。 1例は化学療法により免疫不全状態にあった重症急性リンパ性白血病の9歳女児で、HBVワクチンにより低レベルの抗HBs抗体を獲得していたが、赤血球輸注から13ヵ月後に急性B型肝炎を発症した(発症までの間、全てのHBVマーカーは陰性)。感染原因は、供血血液中にHBV DNAが存在し、供血者と受血者の遺伝子配列(pre-S/SとBCP/PC)の同一性から、輸血による感染と確認された。 もう1例は化学療法による免疫不全状態にあった骨髄異形性症候群の65歳女性(HBs抗原、Hbc抗体陰性、抗HBs抗体低レベル陽性)で、先の症例と同じ供血者から得た血小板の輸注を受けていたが、感染はしなかった。 両受血者の当該輸血前の低レベル抗HBs抗体の存在は、ウイルス量がHBsAg検出限界であると考えられる1000~3000コピー/mL未満と低いため、保護的役割(感染成立の阻害)を果たした可能性がある。また、両受血者は偶然に別の供血者からの高力価抗HBs抗体を含有する血漿又は血小板を輸注されており、HBVの受動抗体を受けていた。両受血者の違いは、1例目が輸血当日に受動抗体を受けたのに対し、2例目は感染源の血小板を投与されてから3日後に受動抗体を受けたことであった。 比較的小量のHBVに曝露した両受血者では、HBVワクチン接種または受動免疫化による予防、あるいは逆に化学療法や免疫抑制による易感染性などの人的介入により、ウイルス感染の早期段階を古典的に定義する変数がかなり修飾されることが示された。この結果、本報告のように複雑な条件下では、輸血、再燃および院内要素を分けて考える必要があり、その解明には進歩した分子方法が最も有用である。					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、・・・陰性で、かつALT (GPT) 値でスクリーニングを実施している。さらに、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。
報告企業の意見		今後の対応			
後にHBV DNA陽性と判明した血液によるB型肝炎感染の報告である。 当社血漿分画製剤の製造工程におけるHBVのモデルウイルスに対するウイルスクリアランス指数は9以上である。なお、原料血漿はミニプール血漿におけるNAT検査でHBV DNA陰性を確認しており、最終製品においてもHBV DNA陰性を確認している。		今後ともにB型肝炎ウイルス感染に関する安全性情報に留意していく。			

A probable case of hepatitis B virus transfusion transmission revealed after a 13-month-long window period

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TRANSFUSION COMPLICATIONS

BACKGROUND: Transfusion-transmitted hepatitis B virus (HBV) infection in recipients with drug-related immunodeficiency is rarely described in endemic areas. Hepatitis B surface antigen (HBsAg)-negative infectious donor blood can be identified by sensitive nucleic acid testing (NAT). Two immunodeficient patients who received blood components from a single seronegative blood donor subsequently found to contain HBV DNA are described.

MATERIALS AND METHODS: Multiple samples from the implicated donor and the two recipients were tested for HBV serologic and molecular markers. HBV genome fragments were amplified, sequenced, and phylogenetically analyzed.

RESULTS: The implicated donation had low-level HBV DNA due to the donor being in the window period before the donor's seroconversion. Recipient 1 had been vaccinated to HBV and carried anti-HBs but remained negative for all other HBV markers until she developed acute hepatitis B (viral load 2.7×10^6 IU/mL and alanine aminotransferase [ALT] level 1744 IU/L) 13 months after transfusion of red cells. Identical HBV sequences from both donor and recipient provided evidence of transfusion-related infection. Recipient 2, who received platelets from the same donation while receiving major chemotherapy, remained uninfected.

CONCLUSIONS: In unusual circumstances, HBV incubation time can be considerably prolonged. Both active and passive neutralizing antibodies to HBV likely delayed, but did not prevent, acute infection when the immune system was impaired. HBV NAT may have identified the infectious unit, although the donation viral load could not be quantified and odds of detection calculated.

Among blood-borne viruses of major concern in transfusion, hepatitis B virus (HBV) presents the highest residual risk,¹ despite several serologic markers available for screening. HBV DNA testing is routinely performed in Germany² and Japan³ and, more recently, in several additional European countries.⁴ HBV DNA testing is an expensive alternative to anti-HBc in place for years in several low-prevalence countries but remains cost-prohibitive in areas of higher prevalence to avoid blood shortage. Genomic screening can be performed on individual donations or in plasma pools ranging between 6 and 96, although it was shown that pooling reduces significantly the yield of DNA-containing donations.⁵ In Brazil, despite relatively high prevalence of the marker, anti-HBc screening is mandatory and a few blood banks also routinely test blood donations for both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) RNA but not for HBV DNA.⁶ A fundamental limitation of anti-HBc screening is the inability to detect window-period, highly infectious, donations. The pre-seroconversion window period has been extensively studied in serial plasma donor samples and typically ranges between 37 and 87 days (median, 59 days).⁷ Post-transfusion infection was not systematically investigated but the early stages were assumed to be of similar or shorter duration due to the large volume of the inoculum. The protective effect of anti-HBs has been well established as well as the increased susceptibility to HBV infection of

ABBREVIATIONS: BCP = basic core promoter; PC = pre-core.

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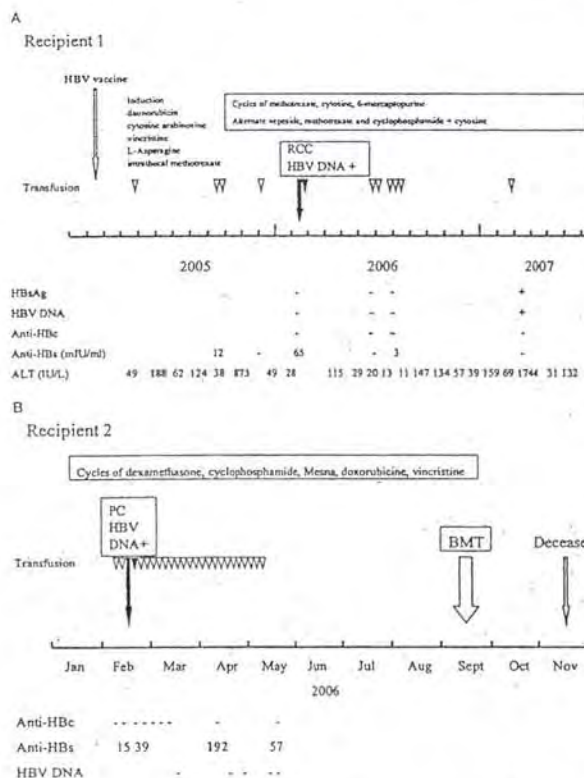


Fig. 1. Case description. (A) Summary of Recipient 1 clinical history. The implicated transfusion of RBCs is indicated by a full arrow. Other transfusions received are indicated as open triangles. The filled triangle indicates the blood product containing high titer of anti-HBs. Bolded ALT levels indicate values above 5 times upper normal level. The HBV infectious component and the PLTs containing high anti-HBs level were transfused on the same day. (B) History of Recipient 2. Symbols are as in Recipient 1 (A). This patient received a PLT concentrate (PC). The interval between receiving the infectious PC and the PC containing high anti-HBs was 3 days. BMT = bone marrow transplantation.

immunodeficient recipients of organs from anti-HBs-carrying donors.

Here are presented two cases of immunodeficient recipients of blood components from a single unit containing very low levels of HBV DNA. One of these recipients developed acute HBV infection 13 months after transfusion despite carrying vaccine-induced anti-HBs while the other was not infected.

CASE REPORT

On March 6, 2007, the hospital notified the blood center that a 9-year-old female child suffering from a high-grade acute lymphoblastic leukemia (Recipient 1), diagnosed in April 2005, was experiencing a clinical episode of acute hepatitis B. Serologic tests confirmed this diagnosis: the presence of hepatitis B surface antigen (HBsAg) and anti-HBc immunoglobulin M (IgM) and an alanine aminotransferase (ALT) level of 1744 IU per L later supported by an HBV DNA load of 2.7×10^8 IU per mL. The patient history revealed 24 transfusions including 13 units of red cell (RBC) and 11 apheresis platelet (PLT) concentrates between April 26, 2005, and August 13, 2006 (Fig. 1A). During this period, she received chemotherapy according to the PROPII-97 protocol consisting of induction by daunorubicin, cytosine arabinoside, vincristine, dexamethasone, and L-asparaginase as well as intrathecal methotrexate/dexamethasone/cytosine-arabinoside. Maintenance treatment consisted of alternate cycles of high-dose methotrexate and cytosine with 6-mercaptopurine, followed by alternate cycles of vepesid plus methotrexate and cyclophosphamide plus cytosine.

Records from the implicated donors were examined and most were excluded as the source of HBV infection because at least one subsequent donation was negative for the presence of HBsAg and anti-HBc. One donor, however, whose RBCs were transfused to the child on February 23, 2006, also donated PLTs by apheresis on March 30, 2006, and subsequent testing results indicated a seroconversion to anti-HBc, without detectable HBsAg, anti-HBs, or HBV-DNA.

A plateletpheresis concentrate prepared from the index automatic blood donation of February 23 (Trima, Gambro BCT, Lakewood, CO) was transfused to a second patient (Recipient 2), a 65-year-old female diagnosed with high-risk myelodysplastic syndrome evolving to biphenotypic leukemia. At the time of the suspect transfusion, she was receiving Hyper-CVAD (ondosetin, dexamethasone, cyclophosphamide, Mesna, doxorubicin, and vincristine) plus intrathecal QT (meth-

Date of sample collection	HBsAg	Anti-HBc* sample OD/cut-off	Anti-HBs	HBV DNA
February 17, 2006	Negative	Negative	ND	ND
Repository samples February 17, 2006	Negative	Negative (0.866/0.407)	ND	Positive
March 31, 2006	Negative	Negative (0.142/0.382)	Negative	Negative

* Hepanostika anti-HBc Uniform, BioMérieux, Boxtel, the Netherlands. Specificity in package insert is 99.85 percent. OD = optical density; ND = not done.

otrexate and aracytin). She was negative for the presence of HBsAg and anti-HBc but had a low level of anti-HBs (13 mIU/mL). In September 2006, she received marrow transplantation in another hospital where no clinical or laboratory evidence of HBV infection was observed. She died of sepsis in November 2006.

Unfortunately, when retrospective investigation was initiated, the archive sample of the implicated donation had already been discarded from the repository according to the national policy mandating the storage of a sample from nonreactive donations for 1 year. Two separate aliquots of 230 μ L of plasma, however, had been archived for potential investigation, allowing us to perform polymerase chain reaction (PCR) amplification and DNA sequencing for comparison with recipient data.

MATERIALS AND METHODS

Serologic testing

Anti-HBc (Abbott/Murex, Delkenheim, Germany), HBsAg (AxSYM MEIA, Abbott Laboratories, Abbott Park, IL), and anti-HBs (AxSYM MEIA, Abbott) testing was performed according to the manufacturer's instructions. Anti-HBs levels are expressed in mIU per mL.

Molecular testing

DNA was extracted from 200 μ L of serum and/or plasma with a DNA blood mini kit (QIAamp, Qiagen, Hilden, Germany) in Brazil and either tested locally or shipped to the UK in dry ice. HBV DNA was detected initially by one-step PCR using 7 μ L of extract DNA submitted to a fast PCR protocol (Applied Biosystems, Foster City, CA) in the presence of 1 μ M of each primer OY1 sense (5'-CAAGGTATGTTGCCCGTTTG-3') and OY2 antisense (5'-AAAGCCCTGACCACTGA-3').⁸ In a final volume of 25 μ L. Nested PCR was performed on 12.5 μ L of DNA in a 25- μ L reaction (final volume) as previously described.⁹ All PCR procedures were performed in a thermocycler (Model 9700, Applied Biosystems). Two nested PCR procedures were used to amplify a 276-bp fragment located in the basic core promoter (BCP) and precore (PC) regions and a 1434-bp fragment spanning the whole pre-S/S gene, as previously described.¹⁰ Sequences of BCP/PC and pre-S/S regions were obtained by direct sequencing of amplicons.

Sequences were aligned with reference HBV genotype A to H sequences using computer software (Clustal W software implemented in Mac Vector Version 7.2, Accelrys, San Diego, CA), and the alignments were confirmed by visual inspection. Phylogenetic analysis was performed using computer software (PAUP 4.0b10, Sinauer Associates, Inc., Sunderland, MA) after exclusion of positions containing an alignment gap from pairwise sequence comparisons. Nucleotide distances were analyzed by neighbor-joining algorithm based on Kimura two-parameter distance estimation. To confirm the reliability of the phylogenetic trees, bootstrap resampling was performed for each analysis (1000 replicates).

RESULTS

Analysis of the implicated donation sample and donor

Upon retesting, the repository sample gave the same serologic results as in the screening (anti-HBc and HBsAg nonreactive) but HBV DNA was detected by two distinct PCR methods, both single-step and nested PCR. The first assay has a limit of detection of 500 IU per mL and the second of 100 IU per mL, and both showed clear amplification, suggesting that, although not properly quantified, the viral load was above 500 IU per mL. Viral load, however, could not be quantified due to the limited sample availability. Of note, the patient and the donor samples were processed 3 weeks apart, the donor sample first, and were kept in different freezers, limiting considerably the possibility of cross-contamination. On the basis of phylogenetic analysis of the pre-S/S gene, the sample was classified as genotype A1. Translation of the "a" region of the S gene indicated a wild-type amino acid sequence when compared to the genotype consensus sequence. The BCP/PC region was also wild type without mutation in either the 1762 to 1764 doublet or the 1896 nucleotide of PC codon 18 or in any of the start codons for PC or core sequences.

When retested from a sample collected 6 weeks after the index donation, the donor plasma showed clear anti-HBc seroconversion but no HBsAg or anti-HBs detectable (Table 1). Other HBV serologic markers such as IgM anti-HBc could not be tested for lack of available sample volume.

The donor was a 39-year-old male who denied risk factors. He was of mixed race, partly of African origin. His donation did not react for anti-HIV and anti-HCV.

Recipient 1

A summary of the Recipient 1 data is presented in Fig. 1A. Before transfusion of the implicated component, anti-HBs was present at low levels on two occasions as expected in a child previously vaccinated to HBV. ALT levels were fluctuating around upper normal levels except on two occasions in May and October 2005 and 2006 when levels reached 188 and 873 IU per L. In the subsequent absence of markers of HBV infection, these high ALT levels could be attributed to the underlying disorder and the chemotherapy. In the period after the transfusion of the implicated component, HBV DNA or serologic markers were never detected until the acute HBV infection 13 months later. During this period, as in the preceding year, ALT levels fluctuated but did not exceed four times upper normal levels. Between transfusion in February 2006 and the acute episode in March 2007, the patient received seven blood components. A single dose of PLT concentrate obtained from a double unit of PLTs prepared by apheresis containing an anti-HBs titer of greater than 1000 mIU per mL was transfused on February 23, 2006, the same day as the implicated HBV DNA containing RBCs. The amount of plasma transfused with the PLTs was approximately 125 mL.

Seven samples collected from Recipient 1 between February 2006 and August 2006 did not contain detectable HBV DNA. After a period of 7 months without transfusion, a sample collected on March 30, 2007 contained a viral load of 2.7×10^4 IU per mL. This strain was sequenced in the BCP/PC and pre-S/S regions. The latter sequence was phylogenetically analyzed and revealed a genotype A1. When these sequences were aligned with the corresponding sequences obtained from the suspected donation, the 276- and 1202-nucleotide-long sequences, respectively, were identical except for one ambiguity. Within the pre-S/S region, Sample SL167648 (donor) showed a sequence ambiguity (adenosine/guanine) at nucleotide 231 starting from the ATG of the S protein. This suggested the presence of quasispecies in the donor while at position 231 only guanine was detected in the recipient sequence. Phylogenetic analysis of the pre-S/S region showed that recipient and donor sequences clustered with HBV genotype A1 reference sequences of African origin, supported by bootstrap values of 100 percent over 1000 replicates. On that basis, the relationship between donor and recipient HBV infection was clearly established. Since HBV genotype A1 in Brazil is essentially found in Brazilians with African ancestry, racial origins of donor and recipient were examined. The donor was of mixed African origin and the recipient was Caucasian.

Recipient 2

Recipient 2 received the PLT concentrate prepared from the same donor and donation transfused to Recipient 1. Follow-up samples collected up to June 2006 (3 months after transfusion) did not reveal the presence of any serologic or molecular marker of HBV infection (Fig. 1B). Before receiving the PLT concentrate from the suspected blood unit, a low titer of anti-HBs was detected acquired either from active or from passive immunity to HBV. The elevation of anti-HBs titer to 192 mIU per mL observed in April 2006 was probably related to passive immunization since, coincidentally, the second unit of a double-plateletpheresis concentrate collected from the same strongly anti-HBs-reactive donation (>1000 mIU/mL) whose PLTs were transfused to Recipient 1 was transfused to Recipient 2. This concentrate contained approximately 125 mL of plasma and was transfused 3 days after the implicated PLT concentrate. Overall, despite receiving PC from an infectious blood donation, no evidence of HBV infection was found in this immunosuppressed adult patient to date.

DISCUSSION

Posttransfusion viral infection has been the focus of considerable scrutiny after the occurrence of HIV infections related to transfusion. Although receiving considerably less attention, reporting of HBV posttransfusion infection has been limited by screening for specific HBV markers such as HBsAg and anti-HBc in some low-prevalence countries. More recently, genomic screening for HBV has become available and was implemented in several countries either in pools of plasma from blood donations or in individual donations. Most anti-HBc screening countries, however, do not feel that it is necessary to screen for HBV DNA and hence do not address the risk of window period. Countries where HBV infection is relatively high (European Mediterranean countries or Poland) as well as some relatively affluent countries with high infection prevalence (Southeast Asia) started screening for DNA to avoid deferring a number of donors that would endanger the blood supply to patients.

Few studies describe the duration of the window period in humans. Most investigate blood donors where the origin of the infection was mostly unknown or post-transfusion. The latter situation had the peculiarity of a large volume of inoculum (100-250 mL) compared to no more than 5 mL in the situation of intravenous drug use, nosocomial infection, or vertical or sexual transmission. In a study conducted in the 1950s, inmates were inoculated with Australian antigen-positive serum; the interval between infection and detection of HBV antigen was 45 to 92 days (mean, 77 days) but longer when the inoculum was diluted 1:1000 (92-130 days).¹¹ The infectious dose seems therefore to influence the duration of the window

period. Other elements possibly interfering in the time interval between viral contact and seroconversion to HBsAg (window period) such as the state of the immune system of the infected individual or the presence of specific neutralizing antibodies to HBsAg have not yet been systematically examined. Only in the situation of transplantation of organs from donors carrying anti-HBs with or without detectable HBV DNA was evidence of infection provided in patients receiving immunosuppressive drugs for liver transplantation.¹² In contrast, experiments conducted in chimpanzees indicated that, in immunocompetent animals, low levels of HBV in the presence of anti-HBs were not infectious.¹³ It has also been well known for many years that the risk of developing chronic HBV infection was inversely proportional to the immunocompetence of children.¹⁴ In none of these circumstances, however, was the duration of the window period or the level of preseroconversion viral load addressed.

In the complicated and discrepant cases presented here, several areas of uncertainty require discussion. First is the authentication of the donation as source of Recipient 1 infection and as a window-period donation. This implication is based on two main elements: 1) the presence of HBV DNA in the donation and 2) the identity of pre-S/S and BCP/PC sequences between donor and recipient. The presence of HBV genome in the implicated donation was found in two separate laboratories in Brazil and in England using different amplification methods and targeted regions. These positive results are strongly supported by obtaining sequences from two such regions. The hypothesis of laboratory contamination is unlikely because the prevalence of chronic hepatitis is 0.2 percent in blood donors in the São Paulo blood center (limiting the possibility of sample to sample cross-contamination) and amplification of HBV in the donor and recipient samples was performed 3 weeks apart from samples stored in different freezers. Finally, being of genotype A1 in a donor of partial African origin is the most plausible since in an unpublished study of 33 strains of HBV from the same blood center, 52 percent of strains were of genotype A1 (J.P. Allain and M. Premnath, unpublished). This dominance of genotype A1 was confirmed by several other studies in Brazil.^{15,16} The donor seroconversion to anti-HBc 42 days after the implicated donation without anti-HBs or HBsAg is not totally convincing (Table 1). While HBV DNA as sole evidence of HBV recent infection strongly suggests being in the window period, the negativity of HBV DNA, HBsAg, and anti-HBs in the second sample is unexpected, unless the stage of infection in the follow-up sample corresponds to the second window period, after disappearance of HBsAg and possibly DNA before the occurrence of anti-HBs. Unfortunately, no further sample was obtained from this donor.

While the identical sequence of more than 1500 cumulated bases between donor and recipient HBV

strains leaves little doubt about the donor being responsible for the infection, once contamination of the donor sample has been excluded, the discrepancy of the outcome of HBV contact between the two recipients raises multiple questions. Although both patients received chemotherapy accompanied with assumed substantial immunosuppressive effects and similar volumes of HBV DNA-containing plasma (110 and 180 mL for Recipients 1 and 2, respectively), only Recipient 1 developed infection. Neither age nor volume of the inoculum could significantly affect the ability to develop an immune response since, at age 9, the maturity of the immune system is comparable to that of an adult. The presence of low levels of anti-HBs before the implicated transfusion in both recipients might have played a protective role, particularly as the blood component viral load was low, below 1000 to 3000 copies per mL, which is considered the limit of detection for HBsAg.^{17,18} Coincidentally, both recipients received passive antibodies to HBV in the form of 125 mL of plasma containing high-titer anti-HBs from the same double-plateletpheresis donation. One difference between the two patients was that Recipient 1 received 125 mL of this plasma the day of transfusion with the implicated product while Recipient 2 received the same volume of plasma 3 days after being in contact with the implicated PLT concentrate. Since the suspected viral strain was wild type in the S region, there is a high likelihood that anti-HBs either raised by vaccine or passively transmitted was neutralizing the circulating virus.

Recipient 1 did not receive any transfusion during the 7 months preceding the episode of acute hepatitis B and, therefore, no reinforcement of her low level of anti-HBs. During the same period of time, the immunosuppressive effect of the chemotherapy accumulated and one can speculate that at one point, the precarious protection offered by low-level neutralizing antibodies became insufficient to contain the virus that started actively replicating.

Posttransfusion HBV infection window period typically ranges between 37 and 87 days in HBV-only infection and between 80 and 110 days when HCV coinfection was present.⁷ The prolongation of the interval between infectious contact and evidence of active viral replication in Recipient 1 was unexpected and remains difficult to explain. Conflicting factors are at play. First the chemotherapy received by the patient to treat leukemia had likely some immunosuppressive effect, which was expected to shorten the window period and facilitate viral replication. In contrast, prior HBV vaccination and passive immunization was expected to prevent or at least delay the clinical expression of the infection. One hypothesis to explain the evidence is that most of the virus received by transfusion was complexed by neutralizing antibodies either actively acquired by vaccination or passively transmitted. Some free virus, however, may have persisted in the liver, escaping the immune system until the level of immunodeficiency

ciency was such that viral replication could take place. This hypothesis is compatible with the surprising absence of detectable HBV DNA in the middle of this long window period in two samples collected in July and August 2006, 5 and 6 months after the infectious contact. Typically, after the eclipse period of approximately 2 weeks during which no evidence of viral DNA is found, low levels of HBV DNA without detectable HBsAg are detectable during the window period.^{17,18,20} Recently a very similar case to ours was published, reporting a 19-week window period in a leukemia patient receiving unspecified chemotherapy regimen and carrying anti-HBs passively transmitted by PLT transfusion (58 mIU/mL) at the time of receiving the low-viral-load window-period donation.²¹

In view of these inconsistencies, the hypothesis of an HBV reactivation from a previously recovered HBV infection can be formulated. Strains mutated in the antigenic "a" region of the S gene, however, are usually found together with anti-HBc.²² In this case, the absence of detectable anti-HBc and the wild-type genotype A1 (Recipient 1 was Caucasian) of the sequenced strain are strong argument against such hypothesis.

These two recipients in contact with a relatively low amount of HBV illustrated that human intervention, whether preventive such as HBV vaccination or passive immunization or to the contrary facilitating infection such as chemotherapy or immunosuppression can considerably modify the variables classically defining the early stages of a viral infection. As a result, in complicated situations such as described here, advanced molecular methods can be most helpful to resolve cases where transfusion, reactivation, and nosocomial elements may need to be separated.

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一般的名称	人全血液	Sato K, Iwata-Takakura A, Yoshikawa A, Gotanda Y, Tanaka T, Yamaguchi T, Mizoguchi H, Vox Sang. 2008; 95(3): 174-80.	公表国	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)	研究報告の公表状況	日本	
研究報告の概要	○B型肝炎ウイルス(HBV)DNAおよびHBV表面抗原の新規濃縮方法:オカルトHBV感染検出方法への応用 背景:輸血後B型肝炎ウイルス(HBV)感染のリスクは、HBV核酸増幅技術(NAT)の導入後減少したが、HBV DNA陽性かつ表面抗原(HBsAg)陰性オカルトHBV感染の問題は未解決である。その理由の一つは、オカルトHBV感染はミニプールNATにより検出するにはHBV DNA量が少なすぎることである。HBVコア抗体(HBcAb)の検査は、オカルトHBV感染を完全に排除していない。そのため、検出感度を上げるために、HBV DNAとHBsAgを同時に濃縮する新規方法を開発した。 方法:二価金属存在下でpoly-L-lysineを使用し、ウイルス凝集反応を増強させ、ウイルスを濃縮する。濃縮処理時間を短縮するためにpoly-L-lysineでコートした磁気ビーズ法を用いる。HBcAb陽性およびHBsAg陰性供血液77本について、酵素免疫法(EIA:AxSYM, Abbott社)および赤血球凝集阻害検査(日本赤十字社)により、HBsAgおよびHBcAbをそれぞれ調べた。 結果:HBV DNAとHBsAg量は、最高4〜7倍に濃縮された。この方法により、HBcAb陽性およびHBsAg陰性供血液77名のうち35名は個別NATにてHBV DNA陽性となり、更に供血液5名はHBVの濃縮によりHBV DNA陽性となった。オカルトHBV感染者40名は個別NATにてHBV DNAおよびHBsAgを濃縮する我々の新しい方法により、EIAとHBV NATの感度が上昇し、HBsAg EIAを用いてオカルトHBV感染者40名のうち27名を検出することができた。			
報告企業の意見	HBV DNAとHBsAg量を、同時に最高4〜7倍に濃縮することで、EIAとHBV NATの感度が上昇し、HBV DNA量が少ないオカルトHBV感染者40名のうち27名を検出することができたとの報告である。			
今後の対応	日本赤十字社では、HBsAg抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HBV検査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。			
使用上の注意記載状況・その他参考事項等	人全血液-LR「日赤」 照射人全血液-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			

MedDRA/J Ver.11.0J

ORIGINAL PAPER

A new method of concentrating hepatitis B virus (HBV) DNA and HBV surface antigen: an application of the method to the detection of occult HBV infection

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Vox Sanguinis

Background The risk of post-transfusion hepatitis B virus (HBV) infection has been reduced after the implementation of HBV nucleic acid amplification technology (NAT). However, the problem of HBV DNA-positive and HBV surface antigen (HBsAg)-negative occult HBV infections remains to be solved. This is in part due to the HBV DNA load being too low to detect these occult HBV infections using mini-pool NAT. In Japan, the assay for the antibody against the HBV core antigen (anti-HBc) has not completely excluded occult HBV infection. To solve this problem, we have developed a new method of concentrating HBV DNA and HBsAg simultaneously to increase the sensitivity of detection tests.

Methods Virus concentration is achieved by the enhancement of the agglutination of viruses using poly-L-lysine in the presence of a bivalent metal. Poly-L-lysine-coated magnetic beads are used to shorten the time of each step of the concentration procedure. Seventy-seven anti-HBc-positive and HBsAg-negative donations were examined. HBsAg and anti-HBc were tested by enzyme immunoassay (EIA) (AxSYM; Abbott) and haemagglutination inhibition test (Japanese Red Cross), respectively.

Results HBV surface antigen and HBV DNA levels were concentrated up to four- to sevenfold. Using this method, 35 of the 77 anti-HBc-positive and HBsAg-negative donors were HBV DNA-positive by individual NAT and a further five donors became HBV DNA-positive by HBV concentration. Twenty-seven of 40 occult HBV infections became HBsAg-positive by HBsAg concentration.

Conclusion Our new method of concentrating HBV and HBsAg increased the sensitivities of EIA and HBV NAT, and enabled us to detect 27 of 40 occult HBV infections by HBsAg EIA.

Key words: anti-HBc, concentration of HBV DNA, concentration of HBsAg, occult HBV infection, poly-L-lysine-coated magnetic beads.

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Introduction

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) [1]. HBV is one of the

most important viral infections transmitted by transfusion. Nucleic acid amplification technology (NAT) screening has widely been introduced for hepatitis C virus (HCV) and human immunodeficiency virus, and has greatly reduced the risk of transfusion-transmitted infection by these viruses. In contrast, HBV NAT has not been widely implemented, in part due to assay sensitivity issues. HBV therefore remains a source of post-transfusion infection. The risk of post-transfusion HBV infection has been reduced after the implementation of

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HBV NAT in Japan, and other countries reduce the risk of transmission by using assays with increased sensitivity for the detection of HBV surface antigen (HBsAg) [2-8]. These approaches have reduced the window period in the early stage of infection. The problem of occult HBV infection, recently defined as individuals who are HBsAg-negative and HBV NAT-positive regardless of the presence or absence of antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs), however, remains to be solved. Anti-HBc screening of blood donations has reduced the risk of occult HBV infection [9-13]. However, in HBV endemic areas such as Asia, anti-HBc screening is not generally utilized, because the rate of positivity is so high that many blood products would be discarded. One possible solution to this problem is to modify the cut-off value of the anti-HBc test and also to take into account the titre of anti-HBs. Using this approach, the Japanese Red Cross (JRC) has succeeded in reducing the frequency of post-transfusion HBV infections, particularly post-transfusion fulminant HBV infection [14, 15]. However, the problem of occult HBV infection has not been completely removed and each year a number of cases of transfusion-associated HBV continue to be reported [16, 17]. In an attempt to address this, the cut-off value of anti-HBc has been decreased and the sensitivity of HBV NAT testing increased by reducing the pool size from 50 to 20 and also increasing the input volume for the NAT assay from 0.2 ml to 0.85 ml [15]. However, there are limitations for the strategy from the view point of cost-effectiveness.

We have developed a new method of concentrating HBsAg and HBV, which could improve the detection of occult HBV infection. The principle of virus concentration is to induce the agglutination of viruses and poly-L-lysine in the presence of a bivalent metal. Poly-L-lysine-coated magnetic beads are used to shorten each step in the concentration procedure.

Materials and methods

Samples

Hepatitis B virus surface antigen-positive and/or anti-HBc-positive donations that did not meet standard JRC requirements were collected with the cooperation of blood centres in the eastern part of Japan from March 2003 to June 2006. None of these donations were used for transfusion purposes. Two hundred and fifty-nine donations were available. These were subdivided into 2.5-ml tubes and stored at -20°C. The remaining plasma from the donation was also stored at -20°C. Of the 259 donations, 182 were HBsAg-positive by enzyme immunoassay (EIA) (AxSYM*; Abbott Laboratories, North Chicago, IL, USA) and 77 were anti-HBc-positive ($\geq 2^3$ by haemagglutination inhibition assay [HI], JRC in-house), HBsAg-negative (EIA; AxSYM*) and anti-HBs-negative ($< 2^4$

[less than 200 mIU/ml]) by passive haemagglutination assay (JRC in-house). An anti-HBc titre $\geq 2^3$ by HI is equal to $\geq 2^7$ -fold diluted sample that is positive ($\geq 50\%$ inhibition) by anti-HBc EIA (AxSYM*).

The 77 anti-HBc-positive donations were used to study the efficacy of the HBV DNA and HBsAg concentration techniques.

Preparation of poly-L-lysine-coated magnetic beads

COOH magnetic beads (125 mg/2.5 ml) (IMMUTEX-MAG™; Japanese Synthetic Rubber, Tokyo, Japan) were added to 0.1 M 2-morpholinoethanesulphate (MES) (Wako Pure Chemical, Tokyo, Japan) solution (final volume, 5.0 ml; pH 5.0) and were incubated for 10 min. Activated magnetic beads (25 mg/ml) were suspended in a coupling buffer [5 ml of 100 mM MES (pH 5.0), 50 µl of 100 mg/ml poly-L-lysine (Wako) and 1.2 ml of distilled water] and mixed by continuous inversion at room temperature for 15 min. Then 1.25 ml of 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (Wako) solution was added to the mixture and mixed by continuous inversion at 10°C for 20 h. Then the solution was replaced with 1 M ethanolamine (Wako) to block reactions at 4°C overnight. Poly-L-lysine-coated magnetic beads were washed five times with phosphate-buffered saline (PBS) and stored at 4°C at a concentration of 50 mg/ml.

It takes 3 days to prepare the poly-L-lysine-coated magnetic beads. Initially, the poly-L-lysine-coated magnetic beads were manufactured in house as described above. Subsequently they have been purchased from JSR.

Concentration of HBsAg and HBV DNA

Poly-L-lysine-coated magnetic beads were added to 2 ml of plasma at a final concentration of 1 mg/ml. Then, 30 µl of 1.1 M Zn(COOH)₂ was added to the sample. The resulting mixture was mixed and left to stand for 5 min. The agglutinated HBsAg/HBV DNA and magnetic beads were trapped in a magnetic field (MagicalTrapper™, Toyobo, Tokyo, Japan) and washed twice with PBS to remove impurities. The concentrated HBsAg was eluted with 0.25 ml of 0.4 M ethylenediaminetetraacetic acid (EDTA) solution. The whole volume of the sample was eluted for EIA testing (AxSYM*, Abbott) (effective eightfold concentration). HBV DNA was eluted with 100 µl of 0.4 M EDTA solution and 50 µl or 100 µl was used for individual NAT (10- or 20-fold concentration, respectively). The concentration and elution process takes 30 min.

HBV DNA extraction and quantification

Hepatitis B virus DNA was extracted using an Ex-R&D kit* (Sumitomo Chemical, Tokyo, Japan). HBV DNA was detected quantitatively as described previously [3]. Briefly, to quantify

the HBV DNA, nucleic acid extracts were amplified and titrated by using a sequence-detection system (TaqMan, ABI Prism 7700 Sequence Detector; PE Applied Biosystems, Foster, CA, USA). Quantification of the HBV DNA was calculated from the working curve (10^7 , 10^6 , 10^5 , 10^4 , 10^3 and 10^2 copies/ml) produced by domestic standard samples that were prepared based on the international standard (NIBSC: National Institute for Biological Standards and Control). Calculation was carried out using Sequence Detector version 1.7 (PE Applied Biosystems). The qualitative detection limit was assumed to be 60 copies/ml (95% confidence interval) and quantitative detection limit was assumed to be 100 copies/ml (95% confidence interval).

The AxSYM* HBsAg assay was used for detection of HBsAg. Tests were carried out in accordance with the manufacturer's instructions. A positive result is defined as a signal/noise (s/n) ratio ≥ 2 . Samples with different concentrations of HBsAg were used to assess the effectiveness of HBsAg concentration. High-titre HBsAg samples (AxSYM*; s/n ratio 266) were sequentially diluted 10-fold up to a final dilution of 10 000-fold using normal plasma. Lower low-titre HBsAg samples (AxSYM*; s/n ratio 12) were diluted up to a final dilution of 1000-fold. Samples known to have HBsAg below the level of detection in the AxSYM assay (s/n ratio 1.7) were diluted to a final dilution of 100-fold. The respective diluted samples were then concentrated eightfold as described above.

The parallel translation of linear line of dilution curves caused by HBsAg dilution and concentration was studied, plotting the s/n ratio of the EIA on the vertical axis to the dilution fold of the samples on the horizontal axis in both logarithm scales.

The effect of anti-HBs on HBV DNA concentration was studied by adding anti-HBs obtained from immunized horse serum. The titre of purified anti-HBs was 51 200 IU/l. The volumes of anti-HBs added to the samples were 0 µl, 20 µl (1024 mIU/l) and 35 µl (1792 mIU/l).

The effects of other viruses on HBsAg and HBV DNA concentrations were studied in the presence of parvovirus B19 (non-enveloped DNA virus) or HCV (enveloped RNA virus).

Data shown in the tables represent the average of the results of two or three experiments.

Results

Hepatitis B virus was concentrated quantitatively by our new method in a broad range of HBV DNA loads. However, the efficacy of concentration varied from sample to sample. The efficacy of concentration [measured value/expected value: original $\times$ concentration times] is shown in Table 1. The efficacy of the concentration process decreased from 0.76 to 0.49 as the HBV DNA load increased from 10^3 to 10^6 copies/ml (Table 1).

Table 1 Effect of the concentration method on concentration of HBV DNA samples

Sample no.	Original (copies/ml)	10-fold concentration (copies/ml)	Efficacy of concentration ^a
1	1.6 E + 06	7.8 E + 06	0.49
2	4.2 E + 05	2.1 E + 06	0.50
3	9.0 E + 04	5.7 E + 05	0.63
4	2.2 E + 04	1.6 E + 05	0.73
5	4.6 E + 03	3.5 E + 04	0.76

^aEfficacy = 10-fold concentration (copies/ml)/original $\times$ 10 (copies/ml).

Table 2 Effect of hepatitis B surface antibody (HBsAb) on concentration of HBV DNA

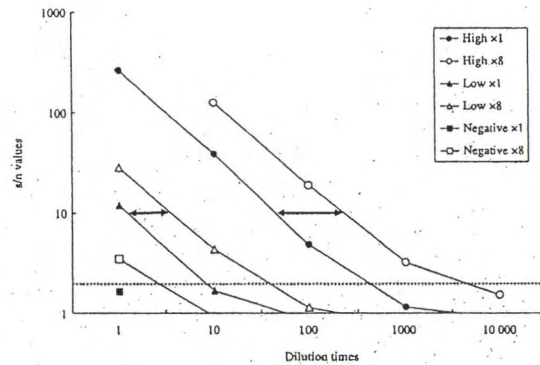
Original sample	10-fold concentration		Efficacy of concentration
HBV DNA (copies/ml)	HBsAb (mIU)	HBV DNA (copies/ml)	
	0	860	0.72
120	1024	1400	1.17
	1792	1300	1.08

The efficacy of HBsAg concentration is shown in Fig. 1. For the high-titre HBsAg samples (s/n ratio 266-03), 100-fold dilution samples were more than limit for detection (s/n ratio 4-88) and 1000-fold dilution samples were less than the limit for detection (s/n ratio 1-16). Following eightfold concentration of HBsAg, the 1000-fold dilution sample was found positive (s/n ratio 3-24). Similarly, in the low-titre sample the undiluted sample was above the detection limit (s/n ratio 11-91). The 10 times dilution sample (s/n ratio 1-69) was negative but became positive following eightfold concentration (s/n ratio 4-36). The negative samples (s/n ratio 1-66) became positive by eightfold concentration (s/n ratio 3-49). Based on the parallel translation of linear line shown in Fig. 1, the relative efficacy of concentration was about 0.64 (5-1/8) in high-titre samples and 0.56 (4-5/8) in low-titre samples.

The effects of anti-HBs and other viruses on HBsAg/HBV DNA concentration were determined. The effect of anti-HBs on HBV DNA concentration is shown in Table 2. The efficacy of HBV DNA concentration in the presence of anti-HBs was superior to that in the absence of anti-HBs. However, in the presence of anti-HBs (antigen-antibody coexistence samples), anti-HBs prevented the detection of HBsAg.

The effect of the coexistence of HCV or parvovirus B19 on the efficiency of HBsAg/HBV DNA concentration is shown in Table 3. HCV (10^6 copies/ml) and parvovirus B19 (2^{11} by RHA: receptor-mediated haemagglutination assay) had no

Fig. 1 Parallel translation of linear line caused by hepatitis B surface antigen (HBsAg) concentration. Vertical axis shows signal/noise (s/n) values of enzyme immunoassay (EIA) indicated by logarithm, and horizontal axis shows dilution fold of samples indicated by logarithm. The linearity was observed more than two (s/n value). Closed circle, high titre of HBsAg ($\times 1$: non-concentration); open circle, eightfold concentration of high titre of HBsAg ($\times 8$: concentration); closed triangle, low titre of HBsAg ($\times 1$: non-concentration); open triangle, eightfold concentration of low titre of HBsAg ($\times 8$: concentration); closed square, negative (s/n; < 2) titre of HBsAg ($\times 1$: non-concentration); open square, eightfold concentration of negative titre of HBsAg ($\times 8$: concentration). The dotted line shows two s/n values (cut-off values). Arrows show the distance of parallel translation by HBsAg concentration.



Data for Fig. 1

		HBsAg: EIA (AxSYM: s/n ¹)				
		dilution with normal plasma				
		1	10	100	1000	10 000
High	$\times 1$	266.03	38.81	4.88	1.16	0.91
	$\times 8$	126.77	18.95	3.24	0.77	0.91
Low	$\times 1$	11.91	1.69	0.86	0.77	
	$\times 8$	28.28	4.36	1.15	0.76	
Negative	$\times 1$	1.66				
	$\times 8$	3.49	0.93	0.8		

Table 3 Effect of coexistence of HCV or parvovirus B19 on efficiency of hepatitis B surface antigen (HBsAg) concentration

Plasma for dilution	AxSYM (s/n ¹)	
	HBsAg dilution with various kinds of plasma ^a	10-fold concentration of diluted HBsAg plasma
Normal plasma	1.39	3.80
HCV-positive plasma ^c	1.18	3.47
Parvovirus B19-positive plasma ^d	1.31	3.77

^aThe original HBsAg-positive plasma titre is 6.19: EIA (AxSYM: s/n).

^bMore than 2 means positive.

^cThe titre of anti-HCV was $> 2^{12}$ and the load of HCV RNA was 10^6 copies/ml.

^dThe titre of B19 antigen was 2^{11} by receptor-mediated-haemagglutination assay.

effects on the concentration of HBsAg/HBV DNA. Although the parvovirus B19 could not be concentrated by this method because of its lack of envelope, HCV RNA could be concentrated quantitatively (data not shown).

Seventy-seven anti-HBe positive ($\geq 2^5$ by HI assay by JRC criteria) and HBsAg-negative (EIA, AxSYM[®]) donations were selected to study the efficacy of HBsAg and HBV DNA concentrations. Of the 77 samples, 35 were positive by individual NAT and a further five became NAT positive

following concentration (Table 4). Of 35 samples (Table 4; lanes d, e), 16 (Table 4; lane e) had HBV DNA loads of 120–1500 copies/ml and the other 19 samples (Table 4; lane d) had HBV DNA loads less than the quantitative detection limit (< 100 copies/ml). However, the HBV DNA loads of all these samples exceeded 100 copies/ml following concentration (Table 4; lanes d, e). Five samples (Table 4; lanes b, c) that were negative by individual NAT became positive (less than 100–510 copies/ml) following concentration.

Table 4 Detection of occult HBV by concentration of HBV DNA and hepatitis B surface antigen (HBsAg)

			HBV DNA (copies/ml)					
			a	b	c	d	e	
Original			Negative	Negative	Negative	< 100	≥ 100	
Concentration (x20)			Negative	< 100	≥ 100	≥ 100	NT	
HBsAg (AxSYM)	I	Original	Negative	34	0	0	8	5
		Concentration (x8)	Negative					
	II	Original	Negative	3	1	4	11	11
		Concentration (x8)	Positive					

NT, not tested.

Of the 40 samples (Table 4; lanes b–e) that were HBV DNA-positive either before or after concentration, 13 were HBsAg-negative even following HBsAg concentration. Of these 13 samples, 5 (Table 4, lane I–e) had HBV DNA loads exceeding 100 copies/ml by conventional individual NAT, and eight (Table 4; lane I–d) were quantitatively less than 100 copies/ml on the non-concentrated sample but became NAT positive (≥ 100 copies/ml) following concentration. Of the 77 samples, 30 (Table 4; lane II) had detectable HBsAg following HBsAg concentration. Of these 30 samples, 27 were NAT positive but three (lane II–a) remained NAT-negative even after concentration. Thirty-four of the 77 samples (Table 4; lane I–a) remained negative for both HBsAg and HBV DNA following concentration for both markers.

Discussion

We have previously reported that HBV DNA could be detected in the HBsAg-negative phases of HBV infection (early window period and occult HBV infections) [2–4, 18]. However, the use of HBV NAT remains limited, because the HBV viral loads seen in HBsAg-negative infected donors (occult HBV infection) are generally low [19–22]. Although the infectivity of occult HBV is low compared to that in the window phases of early infection [17], we have encountered post-transfusion HBV infection caused by both HBsAg and mini-pool NAT-negative, but individual NAT-positive donations [16].

It has previously been reported that NAT sensitivity can be increased by reducing the number of donations in the mini-pool [23], increasing the input volume of serum, and by addition of an ultracentrifugation step [24]. From the viewpoint of cost-effectiveness, an inexpensive and easy method to increase sensitivity is desirable. We have previously reported a virus concentration method using polyethyleneimine [25]. However, HBV DNA and HBsAg were not concentrated qualitatively by the method, because the

combination of extracted nucleic acids of viruses and magnetic beads is difficult to dissociate in the presence of protein-degenerative reagents. We have solved this problem with the use of poly-L-lysine that coagulates with viruses in the presence of bivalent metal ions (zinc acetate).

Owing to the low concentrations of HBV DNA present in early acute infection when both mini-pool NAT and HBsAg are non-reactive, individual NAT would be the best option giving a much higher yield, an increased window period closure, and consequently greater benefit. It is also much debated whether the most sensitive HBsAg detection method is superior to mini-pool NAT, but inferior to individual NAT [21, 23]. If 20-pool NAT samples are concentrated 20 times, the sensitivity of 20-pool NAT might be equal to that of individual NAT.

It is important to determine whether HBV could be concentrated in the presence of anti-HBs. In this study, HBV was much more efficiently concentrated in the presence of anti-HBs than without (Table 2). The results showing that the efficacy of concentration was more than 1.0 might be a result of the easy coagulation of antigen antibody-reacted materials with poly-L-lysine beads. However, in the case of HBsAg concentration, it is difficult to measure the efficacy of HBsAg concentration in the presence of anti-HBs, because anti-HBs inhibits the detection of HBsAg by EIA. The coexistence of other viruses would not affect the concentration of HBsAg/HBV DNA, as shown in Table 3. Moreover, the procedure is useful for concentrating coinfecting enveloped viruses as HCV, although it will be difficult to concentrate non-enveloped viruses as parvovirus B19. HCV that is difficult to concentrate by ultracentrifugation because of its low density is easily concentrated quantitatively by our method.

We succeeded in concentrating HBsAg from occult HBV infection. The theoretical plasma HBsAg concentration was eightfold (2 ml of plasma/0.25 ml of elution); however, from the parallel translation of the linear line (vertical axis = s/n

and horizontal axis – dilution folds of samples), the relative efficacies of concentration were 0.56–0.64. The reason for the low efficacy of HBsAg concentration compared to the efficacy of HBV DNA concentration (0.49–0.76) might be due to HBsAg (22 nm) being smaller than HBV (45 nm) and thus the efficacy of agglutination with poly-L-lysine being different.

In countries where NAT is not available or feasible, the use of a highly sensitive HBsAg assay is crucial in ensuring blood safety. Although individual NAT is the golden standard, at later stages of infection, low concentrations of infectious viruses, which may not be detectable by NAT, might be found in some HBsAg-positive blood donations [19,20]. HBsAg tests with high sensitivity are predicted to have a comparable yield to mini-pool NAT [21]. If the sensitivity of HBsAg detection would be increased by several times, NAT might not always be necessary in late-stage HBV infection. In our study, five samples with low-level HBsAg, detectable only after concentration, were not detected by conventional individual NAT (Table 4; lanes b, c). Twenty-seven of the 40 cases in which HBV DNA was detected were shown to have HBsAg after concentration. The remaining 13 cases (Table 4; lane I–d, e) could not be detected by HBsAg concentration, demonstrating the limitation of our method.

Although HBsAg-negative subjects may retain a low infectivity and have a low risk for progressive liver damage [17], HBV DNA testing or an HBsAg detection method with the highest sensitivity should be implemented to decrease the risk of post-transfusion HBV infection [26,27]. Our new HBV/HBsAg concentration method could contribute to increasing the sensitivity of HBV DNA/HBsAg detection. The concentration method could be combined with either Chemoluminescent Immunoassay (CLIA; PRISM, Abbott) or individual donation NAT to further increase the overall sensitivity of HBV detection. Alternatively, if a high-sensitivity method such as the CLIA was combined with our method, then it might be possible to undertake screening using pooled samples. Our concentration method would potentially be capable of replacing individual NAT by mini-pool NAT, although the present efficacy of concentration is not 1.0 but about 0.7 (Table 1).

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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 2008. 9. 18	新医薬品等の区分 該当なし	総合機構処理欄
一般の名称	解凍人赤血球濃厚液		Aaron S, McMahon JM, Milano D, Torres L, Clatts M, Tortu S, Mildvan D, Simm M. Clin Infect Dis. 2008 Oct 1;47(7):931-4.	公表国 米国	
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)	研究報告の公表状況			
研究報告の概要	○C型肝炎ウイルスの鼻腔内伝播:ウイルス学的および臨床的エビデンス 汚染した薬物吸引器具によるC型肝炎ウイルス(HCV)の鼻腔内伝播の可能性が考えられてはいるが、ウイルス感染源として確定されていない。ニューヨーク市のコミュニティ・クリニックから18歳以上で血液中のHCV PCR陽性の吸引用麻薬常用者38名をリクルーティングした。鼻汁検体を採取したほか、被験者が通常薬物を使用する時のようにストローを使用し、このストローを回収して、血液及びHCV RNAの存在を調べた。鼻汁検体28(74%)、ストロー3(8%)で血液が検出された。HCV RNAは鼻汁検体5(13%)、ストロー2(5%)で検出された。被験者のうち11名では、鼻中隔穿孔など慢性的薬物吸引に関連する鼻の異常が見られた。鼻汁検体と薬物吸引器具に血液とHCV RNAが存在することから、HCV鼻腔内伝播のウイルス学的妥当性が示された。				使用上の注意記載状況・その他参考事項等 解凍赤血球濃厚液「日赤」 照射解凍赤血球濃厚液「日赤」 解凍赤血球-LR「日赤」 照射解凍赤血球-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク
報告企業の意見	汚染した薬物吸引器具によるC型肝炎ウイルスの鼻腔内伝播のウイルス学的妥当性を示したとの報告である。				
今後の対応	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。				

④

Intranasal Transmission of Hepatitis C Virus: Virological and Clinical Evidence

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Intranasal transmission of hepatitis C virus (HCV) via contaminated drug-sniffing implements is a potential but unconfirmed source of viral infection. We demonstrate the virological plausibility of intranasal transmission by confirming that blood and HCV RNA are present in the nasal secretions and drug-sniffing implements of HCV-infected intranasal drug users recruited from a community health clinic in New York City.

Hepatitis C virus (HCV) is the most common bloodborne pathogen in the United States and is a major cause of liver-related morbidity, mortality, and liver transplantation [1]. HCV is transmitted through contact with infected blood [2] (mostly via shared needles and other drug injection paraphernalia); however, a large proportion (up to 20%) of HCV infections remain unexplained, especially among noninjection drug users [3]. One hypothesis to account for these unexplained cases involves intranasal transmission of HCV via contaminated implements, such as straws, used to snort cocaine, heroin, and other powdered drugs [4]. Implements inserted into the nasal cavity, which has been eroded by long-term drug sniffing, might come into contact with HCV-infected mucus or blood, which might then be transmitted to a susceptible individual sharing the same implement [5]. Epidemiological studies of intranasal transmission of HCV have produced inconsistent findings [6,

7], in part because of the high correlation between drug sniffing and other risk factors for HCV infection. Here, we attempt to reduce the intranasal transmission hypothesis by invalidating ≥1 of its virological preconditions. Specifically, we address 2 primary research questions: (1) Does HCV RNA exist in the nasal secretions of serum-positive drug sniffrs? (2) If so, can HCV RNA be transferred onto the sniffing implements shared by intranasal drug users? A secondary aim was to examine clinical nasal pathologies that might facilitate intranasal HCV transmission.

Methods. Our sample included low-income, urban intranasal drug users with chronic, active HCV infection. Subjects were primarily Hispanic and African American and were recruited from a neighborhood health clinic in East Harlem, New York City, an area with a high prevalence of HCV infection (up to 29%) among noninjection drug users [3]. Eligibility criteria included (1) age ≥18 years; (2) self-reported intranasal drug use; and (3) a positive result of a quantitative HCV PCR blood test. Overall, 38 patients enrolled in the study and provided informed consent. Study protocols were approved by 3 institutional review boards.

The following medical information was obtained from subjects: quantitative HCV RNA test result and viral load, hepatitis B antibody test results, liver enzyme levels (i.e., alanine aminotransferase level), and liver biopsy history. Subjects completed a brief survey, in either Spanish or English, that covered demographic characteristics, risk factors for HCV infection, injection and noninjection drug use, health status, and nasal pathology symptoms.

Blood samples were collected for quantitative PCR. Two nasal secretion samples (1 from each nostril) were collected with Dacron nasal swabs and placed in (1) 1 mL of TRIzol reagent (Gibco BRL) for RNA detection or (2) 1 mL of QIAzol lysis reagent (Qiagen) for RNA detection. Similarly, 2 experimental sniffing implements, which consisted of new (packaged) soda straws commonly used by drug sniffrs, were collected from each subject. To avoid harmful effects of sniffing powdered substances, subjects were instructed to "snort air" while mimicking their normal drug-sniffing behavior.

HCV RNA was isolated from 200 µL of serum by use of the QIAamp MinElute kit (Qiagen). HCV RNA was isolated from nasal secretions and sniffing implements using the TRIzol (Gibco BRL) on the basis of established protocols [8]. The first strand of cDNA was synthesized by ImProm-ITM Reverse Transcription System (Promega) using gene-specific downstream primers targeting the HCV p22 core region, with minor

BRIEF REPORT

Table 1. Detection of hepatitis C virus (HCV) RNA and blood in biological specimens obtained from 38 patients with HCV-positive serum specimens.

Assay	No. (%) of persons (n = 38)	95% CI
Blood detection with OBTI	28 (73.7)	57.8-85.2
Nasal secretions	26 (73.7)	57.8-85.2
Sniffing straws	3 (7.9)	2.0-21.5
HCV RNA detection with PCR	5 (13.2)	5.3-27.8
Nasal secretions	5 (13.2)	5.3-27.8
Sniffing straws	2 (5.3)	0.5-18.2

modification of the upstream primer (410R-5'-ATGTACCCCA-TGAGGTCGGC-3'). HCV cDNA was amplified by PCR with 40 cycles of denaturation (94°C for 30 s), annealing (58°C for 30 s), and elongation (72°C for 45 s) with primers 406F-5'-TAGACCGTGACCATGAGC-3' and 410R. PCR products were detected by Southern blot using ³²P-labeled probe (5'-AGGAAGACTTCGAGCGGTGCGAA-3').

HCV cDNA was amplified from randomly selected HCV-positive blood samples with use of high-fidelity Pfu polymerase (Perkin Elmer) using 410R and 406F primers and cloned into a TA cloning vector (Invitrogen). The pTA_{HCV} was used to prepare standard curves ranging from 1 × 10¹ to 10 copies of HCV mRNA, which were run in parallel to each set of samples. The intensity of DNA bands was evaluated by densitometry using the Kodak Image Analysis System; the HCV load for the test sample was calculated on the basis of the numeric value derived from the HCV titration curve. HCV load was calculated as the number of copies per milliliter for blood specimens and as the number of copies per sample for nasal secretions and implements.

Traces of blood in nasal secretions and sniffing implements were detected by Hexagon OBTI Kit (BLUESTAR Forensic). Titration curves were prepared using human hemoglobin (Sigma) in 2-fold dilutions ranging from 10 to 0.1 µg/mL. The concentration of blood in each sample was established by comparing the OBTI intensity between the sample and the hemoglobin titration curve.

Nasal cavity pathology was assessed for each patient by anterior nasal examination, rendering diagnoses on 8 nasal pathologies. Rhinitis was diagnosed on the basis of the classic symptoms of mucosal and nasal secretion appearance [9]. Rhinosinusitis was defined by symptomatic inflammation of the paranasal sinuses and nasal cavity [10].

Sample prevalences of HCV RNA and occult blood in nasal secretions and on sniffing implements were estimated. Ninety-five percent CIs were calculated around point estimates using the adjusted Wald method. Descriptive statistics were calculated for sample descriptors and measures of nasal pathology. Our

limited sample size precluded statistical tests of significance (e.g., associations between virological and clinical variables).

Results. All 38 patients had chronic, active hepatitis C. The serum HCV load ranged from 250 to 5,000,000 copies/mL (median, 5000 copies/mL). Recent liver biopsies had been performed for 6 patients; all indicated chronic liver disease, with stages ranging from 1 to 4. Recent alanine aminotransferase levels were available for 17 patients; the mean level (±SD) was 46.7 ± 26.7 U/L (range, 16–118 U/L). Antibody screening revealed that 34% of subjects were positive for antibodies to HIV, and 45% were positive for antibodies to hepatitis B virus.

Trace amounts of blood were detected in 28 (74%) of 38 nasal secretion samples (range, 0.1–10 µg/mL) and on 3 (8%) of the 38 sniffing implements (range, 0.1–2 µg/mL). HCV RNA was detected in 5 nasal secretion samples (13%; HCV RNA level range, 10–100 copies/sample) and on 2 sniffing implements (5%; HCV RNA level, 50 and 100,000 copies/sample). Prevalence estimates suggest a wide discrepancy between the presence of blood (74%) and the presence of HCV RNA (13%) in the nasal secretion samples (table 1). Of the 5 HCV RNA-positive nasal secretion samples, only 3 had traces of occult blood; of the 28 samples containing occult blood, 25 were negative for HCV RNA (figure 1).

The prevalence of rhinitis in this cohort was high (71%) (table 2). In contrast, the prevalence of rhinosinusitis (11%) is consistent with that of the general population. More than 40% of subjects experienced rhinorrhea or nasal congestion at least once per week, 8% reported nose bleeds at least once per week, and 8% and 16% reported mucosal lesions and crusting, respectively. Approximately one-half of the subjects attributed these symptoms to intranasal drug use. Four persons (11%) were observed to have nasal septal perforations; 1 (3%) had a nasopalatal perforation; and 6 (16%) displayed symptoms of saddle nose deformation. These pathologies have been associated with advanced nasal cavity deterioration associated with chronic intranasal drug use [11].

Discussion. Our findings revealed a high prevalence of blood (74%) in the nasal secretions of HCV-positive long-term drug sniffers. We also confirmed that HCV RNA was present in the nasal secretions of a substantial proportion (13%) of this cohort. Most significantly, this study demonstrated that both blood and HCV particles can be transferred onto sniffing im-

		Occult Blood in Nasal Secretions		
HCV RNA in Nasal Secretions	Pos.	Pos.	Neg.	
	Neg.	3	2	5
		25	8	33
		28	10	38

Figure 1. Hepatitis C virus (HCV) RNA and occult blood in nasal secretions.

Table 2. Frequency of nasal pathology symptoms among intranasal drug users.

Symptom	No. (%) of subjects (n = 38)
Findings of an anterior nasal clinical examination	27 (71.1)
Loss of nasal hairs	4 (10.5)
Rhinitis	27 (71.1)
Rhinosinusitis	4 (10.5)
Presence of nasal crusting and/or scabbing	6 (15.8)
Sores or erosion of nasal mucosa	3 (7.9)
Saddle nose deformation	6 (15.8)
Nasopalatal perforation	1 (2.6)
Nasal septum perforation	4 (10.5)
Self-reported nasal pathology	26 (68.4)
Frequency of nosebleeds in the past year	26 (68.4)
Never, or rarely	9 (23.7)
Once or a few times per month	2 (5.3)
Once or a few times per week	1 (2.6)
Once or more per day	1 (2.6)
Experienced a runny or stuffy nose in the past year	16 (42.1)
Never, or rarely	6 (15.8)
Once or a few times per month	13 (34.2)
Once or a few times per week	3 (7.9)
Once or more per day	3 (7.9)
Reason for nasal symptoms	19 (50.0)
Allergies	10 (26.3)
Cold or influenza	2 (5.3)
Drug sniffing	2 (5.3)
*Have you ever noticed any of the following problems with your nose due to drug sniffing?	
Scabs in the nose	14 (36.8)
Sores in the nose	8 (21.1)
Poor sense of smell	13 (34.2)
Sinus pain	13 (34.2)
Headaches located in the forehead	16 (42.1)
Double vision	5 (13.2)
*Has a doctor or other health care professional ever told you that the inside of your nose is damaged in any way from sniffing drugs?	7 (18.4)

plements (i.e., straws) during simulated intranasal drug use. Studies have shown that HCV can remain viable on environmental surfaces for up to 16 h, but little is known about the quantity of virus required for transmission [12]. The prevalences of HCV in the nasal secretions and on sniffing straws are likely conservative estimates. It is reasonable to assume that HCV will be present in the nasal secretions with greater frequency and quantity during episodes of active drug sniffing, which may exacerbate discharge of nasal fluids and blood.

Data in table 1 contradict the assumption that, in persons with HCV-positive serum specimens, detection of blood implies the presence of HCV. This discrepancy may be explained by 2 factors. First, the 2 assays (PCR and OBTI) were not performed on the same samples. Second, the OBTI assay for blood detects

immune complexes between human hemoglobin (hHb) and monoclonal anti-hHb antibodies, which can occur even in the absence of viable cells. In contrast, PCR can only detect HCV RNA from intact particles. Therefore, the discrepancy between the high prevalence of occult blood and relatively low detection of HCV RNA in nasal secretions may be associated with the rapid deterioration of viral RNA in the nasal environment or the destruction of viral particles by mucosal immunity. If the viability of HCV particles in nasal secretions is moderated by nasal pathology or immunity, this might help explain conflicting epidemiological findings in which these moderating factors are not considered.

This study establishes the validity of 2 primary virological preconditions necessary for intranasal HCV transmission: (1)

the presence of blood and HCV in the nasal secretions of intranasal drug users, and (2) the transference of blood and HCV from the nasal cavity onto sniffling implements, which are often shared by intranasal drug users. Moreover, the frequency and severity of nasal pathologies observed in this cohort might aggravate conditions that facilitate intranasal HCV transmission. Consequently, these findings lend important virological and clinical support to the intranasal HCV transmission hypothesis. In addition, detection of HCV in nasal secretions advances the debate regarding potential iatrogenic and nosocomial transmission of HCV in the context of ear, nose, and throat and related clinical practices. More research is needed to confirm intranasal transmission as a mode of viral infection and to determine its impact on the wider epidemic of HCV infection.

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Potential conflicts of interest. All authors: no conflicts.

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医薬品 研究報告 調査報告書

総合機構処理欄		新医薬品等の区分		第一報入手日	報告日	識別番号・報告回数
使用上の注意記載状況・その他参考事項等		公表国 英国	Vox Sanguinis (2008) 95, 94-100		研究報告の 公表状況	一般的名称 — 販売名(企業名) —
E型肝炎ウイルス(HEV)はヘペウイルス属に分類され、エンベロープがなく、直径約27~34nmのウイルスで、糞便から経口、食物媒介および血液媒介経路で伝播され、ヒトの肝炎の原因となる。 今までの報告では、56℃30分間の加熱で4つのHEV株が不活化され、別の報告では糞便由来の3つのHEV分離株は、56℃または60℃60分の加熱で不活化されたが、熱抵抗性の特性は株間でわずかに異なるとされている。 本研究では、日本で発見された遺伝子型3と4の4つのHEV分離株を用いて、アルブミン及びフィブリノゲンにおける液状加熱、乾燥加熱およびウイルス除去膜ろ過によるHEV不活化/除去能を検討した。 その結果、25%アルブミン(液状加熱段階直前に採取)では、60℃で5時間の加熱を行ったが、いずれのHEV分離株も熱抵抗性を示し、5時間加熱後も感染力が検出され、LRF(log reduction factor)はそれぞれ2.0、2.0、1.0および2.2以上であった。 フィブリノゲン(2.0 w/v%塩酸L-アルギニン含有、乾燥加熱段階直前に採取)では、60℃で72時間処理したところ、感染力が検出された。 また、ウイルス除去膜では、いずれのHEV分離株も、孔径19nmおよび15nmでは検出限界以下まで除去されたが、35nmでは大量のHEVが検出され、HEVの粒子サイズは既に電子顕微鏡分析で報告されているように約35nmであることが示唆された。		報告企業の意見 HEVの不活化、除去に関する情報である。現在まで、血漿分画製剤による伝播の報告はなく、製造工程中には複数のウイルス不活化除去工程を設けているが、今後とも関連情報の収集に努める。		今後の対応 今後ともHEVに関する情報に留意し、関連情報の収集に留意していく。		

ORIGINAL PAPER

Extent of hepatitis E virus elimination is affected by stabilizers present in plasma products and pore size of nanofilters

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Vox Sanguinis

Background and Objective To investigate the physico-chemical properties of hepatitis E virus (HEV) with regard to inactivation/removal, we have studied four isolates with respect to sensitivity to heat during liquid/dry-heating as well as removal by nanofiltration.

Materials and Methods Hepatitis E virus in an albumin solution or phosphate-buffered saline (PBS) was liquid-heated at 60°C for a preset time. HEV in a freeze-dried fibrinogen containing stabilizers was also dry-heated at 60 or 80°C for a preset time. In addition, to clarify the removal of HEV, the purified virus in PBS was filtered using several types of virus-removal filter (nanofilters) that have different pore sizes. HEV infectivity or genome equivalents before and after the treatments were assayed by a semiquantitative cell-based infectivity assay or quantitative polymerase chain reaction assay, respectively.

Results Hepatitis E virus isolates in albumin solutions were inactivated slowly at 60°C for 5 h and the resultant log reduction factor (LRF) was from 1.0 to ≥ 2.2 , whereas the virus in PBS was inactivated quickly to below the detection limit and the LRF was ≥ 2.4 to ≥ 3.7 . The virus in a freeze dried fibrinogen containing trisodium citrate dihydrate and L-arginine hydrochloride as stabilizers was inactivated slowly and the LRF was 2.0 and 3.0, respectively, of the 72 h at 60°C, but inactivated to below the detection limit within 24 h at 80°C with an LRF of ≥ 4.0 . The virus in PBS was also confirmed as to be approximately 35 nm in diameter by nanofiltration. These results are useful for evaluating viral safety against HEV contamination in blood products.

Conclusion The sensitivity of HEV to heat was shown to vary greatly depending on the heating conditions. On the other hand, the HEV particles were completely removed using 20-nm nanofilters. However, each inactivation/removal step should be carefully evaluated with respect to the HEV inactivation/removal capacity, which may be influenced by processing conditions such as the stabilizers used for blood products.

Key words: dry-heating, heat inactivation, HEV, liquid-heating, nanofiltration.

Introduction

Hepatitis E virus (HEV), classified in the genus *Hepevirus*, is a causative agent of human hepatitis. The virus capsid is non-enveloped and the nucleocapsid containing positive-sense single-stranded RNA has a diameter of 27–34 nm [1]. HEV

is also endemic in humans, swine and several wild animals such as deers and boars, suggesting that hepatitis E is a zoonosis [2,3].

The virus has been shown to be transmitted by faecal-oral, food-borne and blood-borne routes [1,4–7]. Four genotypes of HEV that infect humans have been identified, three of which, genotypes 1, 3 and 4, have also been isolated from swine and commercial swine liver [1,8,9]. Zoonotic food-borne transmission of HEV was shown to be one reason for the occurrence of a severe form of hepatitis E in Hokkaido, Japan, and HEV genotype and the presence of an underlying disease influenced the severity of the hepatitis E infection [10]. In addition, the prevalence of HEV RNA or anti HEV immunoglobulin G (IgG)-positive blood donors in Hokkaido was 0.01% (56/432,167) and 3.9%, respectively [11]. These reports also suggested that a small but significant proportion of blood donors in Japan with or without elevated alanine aminotransferase (ALT) levels are viremic and are potentially able to cause transfusion-associated hepatitis E. Note that anti-HEV IgG and HEV levels in pooled plasma have not been reported yet. Thus, these data may indicate the need for precautions against the potential risk of transfusion-transmitted HEV infection, as previously discussed [12]. In addition to foods, the safety of plasma-derived products with respect to HEV may be an important issue and each product should be evaluated for safety against HEV contamination.

Huang *et al.* reported that four HEV strains in culture media containing 2% calf serum were inactivated, and that residual infectivity was not detected after heating at 56°C for 30 min [13]. Emerson *et al.* reported that three HEV isolates derived from faeces including genotypes 1 and 2 were inactivated after 60 min at 56 or 60°C, but the heat-resistance properties differed slightly between the strains used. A strain that was slightly more resistant to heating showed some residual infectivity (<1%) after 1 h at 56°C [14]. Tanaka *et al.* also reported that an HEV isolate in a faecal suspension in Tris-HCl buffer was inactivated and that residual infectivity was not detected after heating at 70°C for 10 min, whereas residual infectivity was detected after 30 min at 56°C [15]. Unfortunately, these studies did not evaluate the log reduction of infectivity and kinetic pattern of inactivation.

There have been no reports of HEV transmission via plasma-derived products that contain various kinds of proteins at high concentrations and also various types of stabilizers. However, investigative methods with log reduction and/or general information on HEV regarding the contamination of blood products have been required. In this study, we investigated the impact on the ability to inactivate HEV during liquid/dry-heating and viral particle removal by nanofiltration in plasma protein preparations using four HEV isolates found in Japan and belonging to genotypes 3 and 4.

Materials and methods

Viral isolates

Isolates from four different HEV clusters were used, that is, genotype 3_{sp} [swJB-E, cluster SP (3e), GENBANK (in preparation by Yamate *et al.*)], genotype 3_{us} [swJB-M, cluster US (3a), GENBANK (in preparation by Yamate *et al.*)], genotype 3_{pe} [swJB-N, unclassified cluster, GENBANK (in preparation by Tsunemitsu *et al.*)], and genotype 4_{pe} [swJB-H, cluster JP (4c), GENBANK (in preparation by Yamate *et al.*)] (Table 1). These viruses were derived from faeces of infected swine in Japan. The origins of swJB-H, swJB-E and swJB-M were naturally infected swine faeces, while swJB-N was from faeces of experimentally infected swine (Highland strain, kindly provided by Dr Hiroshi Tsunemitsu, National Institute of Animal Health, Japan).

Table 1 Details of viral isolates used

Genotype ^a	Isolation ID	Viral titre		Used for
		HEV genome ^b	HEV infectivity ^c	
3 _{pe}	swJB-N2	6.3	3.8	Liquid-heating, nanofiltration
3 _{us}	swJB-M5	7.2	4.8	Nanofiltration, dry-heating
3 _{sp}	swJB-M8	8.4	5.3 ^d	Liquid-heating
	swJB-E8	7.5	4.8	Dry-heating
	swJB-E10	7.7	5.8 ^d	Liquid-heating, nano-filtration
4 _{pe}	swJB-H1	7.0	–	Nanofiltration
	swJB-H1/H7	7.0/7.4	4.8 ^d	Liquid-heating
	swJB-H7	7.4	3.2 ^d	Liquid-heating
	swJB-H8	6.8	3.8	Liquid-heating
	swJB-H21 ^e	7.2	3.8	Liquid-heating

^aThe genotypes and clusters of isolates were grouped as described by Takahashi *et al.* and Lu *et al.* [24,25].

^bGenome amount is indicated by log copies per ml. For swJB-M, specific primer sets and probes (sense primer F2: 5'-TCGTGTACAAACCGAGATTC-3', anti-sense primer R2: 5'-GCCGGGCAATATTGTCTA-3', Probe Flu2: 5'-GATGCAACCCCGCAGTTGGTTTC-3' and Probe LC2: 5'-LCRed640-GCCCTGAGGTACTCTGGAATCATCTATCC-3') were designed and used. For the other isolates, the primer set and probe (HEB6, HEB7 and FAM-labeled probe FHE88) designed by Jothikumar *et al.* [26] were used.

^cInfectivity titre is given as log dilution non-detectable end-point per ml.

^dMean titre of two (three) independent experiments.

^eMixture of H1 and H7 used.

^fThis isolate is derived from faeces of an experimentally infected piglet.

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Isolation and purification of virus

Faecal samples (10 g) were resuspended with 100 ml of phosphate-buffered saline (PBS) and centrifuged at 1600 *g* for 10 min and the supernatant retained. Pellets were resuspended in 50 ml of PBS and the suspension was centrifuged again under the same conditions. Resultant pellets were resuspended with 25 ml of PBS and the suspension was centrifuged again. All these three supernatants were pooled and were filtered using an AP filter (AP2504700, Millipore, Billerica, MA, USA). After centrifugation at 10 000 *g* for 30 min, the supernatant was filtered through four sequential filters (5.0 µm; SMWP04700, 1.2 µm; RAWP04700, 0.8 µm; AAWP04700, and finally 0.45 µm; HAWP04700, Millipore). Then polyethylene glycol (PEG) 6000 (Wako Pure Chemical Industries, Osaka, Japan) and sodium chloride up to final concentrations of 8% (w/v) and 2.4% (w/v), respectively, were added to the final filtrate. The solution was stirred for 10 min and incubated overnight at 4°C. The solution was centrifuged at 10 000 *g* for 30 min and the precipitate was resuspended with one-tenth the volume of the original solution of PBS prior to the addition of PEG. The solution was sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The resultant supernatant was filtered in two steps (0.45 µm; SLHV033RS and 0.22 µm; SLGV033RS, Millipore), and the filtrate was aliquoted and stored at -80°C as HEV stock. Isolated HEV samples were allocated an isolation ID and preparation lot number.

Hepatitis E virus stocks were further purified for filtration experiments. The viral stocks in PBS were treated with 1% (v/v) Tween-80 (Wako Pure Chemical Industries) and 0.3% (v/v) Tri-*n*-butyl Phosphate (TNBP, Sigma, St. Louis, MO, USA) for 1 h at 30°C and then the solutions were ultracentrifuged at 150 000 *g* for 3 h at 4°C. The precipitates were resuspended in PBS and subsequently sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The supernatants were filtered by sequential 0.22 and 0.1 µm filtration (SLGV033RS (0.22 µm) and SLV033RS (0.1 µm); Millipore) and the filtrate was aliquoted and stored at -80°C as purified HEV stock. In addition, HEV Genotype 3_{SP} derived from the culture media of infected A549 cells was treated with detergent alone, as described above, and subsequently used for filtration experiments.

Quantitative HEV RNA assay for each isolate

The total HEV RNA in each sample was extracted using the RNeasy Mini Kit (cat. 74104; Qiagen GmbH, Hilden, Germany) and then quantified by polymerase chain reaction (PCR) using specific primers. The copy number of swJB-M was quantified using specified primers and probes set from the light cycler (LC) RNA Amplification Kit Hybridization Probes (Roche Diagnostics, Basel, Switzerland) and LC quick system 350S (Roche Diagnostics). The assay conditions were as

follows: reagents; 4.0 µl of 5× LC reverse transcription (RT)-PCR Mix HybProbe (Roche Diagnostics), 3.2 µl of 25 mM MgCl₂, 2.0 µl of 5 pmol/µl primer F+R, 2.0 µl of 2 pmol/µl probe Flu+LC, 3.4 µl of water, 0.4 µl of LC RT-PCR enzyme mix and 5.0 µl of template (total 20 µl), and reaction; 55°C 10 min, 95°C 30 second, 45 cycles of 95°C 5 second, 60°C 15 second, 72°C 13 second and subsequently 40°C 30 second. The copy number of ORF3 for swJB-N, swJB-E and swJB-H (genotypes 3_{SP}, 3_{SP} and 4_{SP}) was also quantified using a QuantiTect Probe RT-PCR Kit (Qiagen) and Applied Biosystems 7500 (Applied Biosystems, Foster City, CA, USA). The assay conditions were as follows: reagents; 25 µl of 2× QuantiTect Probe RT-PCR Master Mix (Qiagen GmbH), 1.0 µl of 20 µM primer Mix, 0.5 µl of 10 µM Probe, 0.5 µl of QuantiTect RT Mix, 13.0 µl of water and 10 µl of template (total 50 µl), and reaction; 50°C 30 min, 95°C 15 min, 45 cycles of 95°C 15 second and 60°C 35 second.

Infectivity assay for HEV

Infectivity of HEV was assayed according to Huang et al. [13] with minor modifications. A549 cells (kindly provided by Dr Takaaki Nakaya, Research Institute for Microbial Diseases, Osaka University) were cultured in DMEM (cat. 11995-065, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (cat. SH30071-03; Hyclone, Logan, UT, USA), 100 U/ml penicillin, 100 µg/ml streptomycin (cat. 15140-122, Invitrogen) and Insulin-Transferrin-Selenium-X (ITS-X) supplement (cat. 51500-056, Invitrogen) at 37°C in 5% CO₂ in air. The composition of the medium used for the viral assay was Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum, 100 U/ml penicillin, 100 µg/ml streptomycin, ITS-X supplement and 30 mM MgCl₂ (cat. 135-00165, Wako Pure Chemical Industries) at 37°C in 5% CO₂ in air. For the infectivity assay, A549 cells were seeded in a 12-well microplate (3.6 × 10⁵ cells/ml, 2 ml/well). After an overnight culture, the cells were inoculated with serial 10-fold dilutions of the virus stock solution (0.3 ml/well). On day 7 of culture, HEV RNA in cultured cells was assayed using the HEV RNA assay method described above. The infectivity of each stock of isolate used was determined from the dilution end-point where no RNA was detected.

Heat sensitivity of HEV during liquid- and dry-heating

Hepatitis E virus isolates were ultracentrifuged at 150 000 *g* for 3 h at 4°C. The resultant pellets were resuspended with PBS or a 25% albumin solution that was collected just before the heating step in the manufacture of Kenkitsu Albumin-Wf (Benesis, Osaka, Japan) as a stabilizer. These samples were aliquoted at 0.5 ml per tube and incubated in a water bath at 60°C for preset times (0, 0.5, 1, 2 and 5 h). After quickly

cooling, the residual infectivity of the sample was determined as described above.

The HEV precipitates described above were also resuspended with a Fibrinogen solution containing 1.3% (w/v) trisodium citrate dihydrate and 2.0% (w/v) L-arginine hydrochloride as a stabilizer that was collected just before the dry-heating step in the manufacture of Fibrinogen HT-Wf (Benesis). The HEV solutions were aliquoted at 2.0 ml/vial and freeze-dried using an optimized freeze drying cycle (programme) for this product (freeze dry systems cat. 7948020 and 7934024, Labconco, Kansas City, MO, USA). The freeze-dried samples in the vials were closed under vacuum. The vials were then heated at 60 or 80°C in a drying oven (cat. DK43; Yamato Scientific, Tokyo, Japan) for 72 h. The heated samples were cooled quickly and stored at 4°C until the assaying. Residual infectivity was assayed as described above. In addition, the residual water content of mock-infected samples prepared using the same freeze drier programme and conditions without spiking with HEV were assayed using the loss on drying test method described previously [16].

Removal of HEV by nanofiltration

Hepatitis E virus stocks that were detergent-treated, as described above, were thawed, concentrated, if required, sonicated and filtered using 0.22 µm (0.22 µm; SLGV033RS, Millipore) and Bemberg Microporous Membrane (BMM) filter (Planova®-75N (72 ± 4 nm, 0.001 m²); Asahi Kasei Medical, Tokyo, Japan) immediately prior to nanofiltration. The viral samples were subjected to nanofiltration using BMM-35N (35 ± 2 nm, -20N (19 ± 2 nm) and -15N (15 ± 2 nm; Asahi Kasei Medical) under conditions where 2-ml samples were applied to 10⁻⁵ m² filters with 50 kPa and dead end filtration. The quantities of HEV RNA before and after filtration were measured using the quantitative HEV RNA assay described above.

Results

Viral preparations

Isolates from four different clusters including two genotypes were prepared and each isolate was evaluated regarding genome and infectious titre in the stocks.

We evaluated the appropriateness of the method to determine the HEV infectious titre by semiquantitative PCR (data not shown). The levels of HEV RNA in the infected cells were higher at 3 and 7 days post-infection (dpi) than at 0 dpi. The titres obtained were not consistent on 3 dpi whereas the results were consistent on 7 dpi. Therefore, we decided that the titre of HEV should be determined on 7 dpi. According to our data, about 1000 copies of the genome per infectious unit were observed in our system. The infectious titres in the HEV stocks of the viruses are summarized in Table 1.

Heat sensitivity of HEV

The heat-inactivation kinetics of HEV isolates from four clusters including two genotypes during liquid-heating using 25% albumin and PBS at 60°C for 5 h was evaluated. All isolates in PBS were inactivated below the detectable infectivity limit within 30 min at 60°C and showed a rapid inactivation. The log reduction factor (LRF) of genotype 3_{SP}, 3_{SP}, 3_{US} and 4_{SP} was ≥ 2.7, ≥ 3.7, ≥ 3.7 and ≥ 2.4, respectively. In contrast, all HEV isolates in the 25% albumin solution showed heat resistance, and residual infectivity was detected even in the samples heated for 5 h and the LRF was 2.0, 2.0, 1.0 and ≥ 2.2, respectively (Fig. 1).

The heat-inactivation kinetics of Genotype 3_{US} and 3_{SP} in fibrinogen during dry-heating was also evaluated. The water content of freeze-dried samples containing the two HEVs was < 0.3%. Residual infectivity was not detected with the LRF

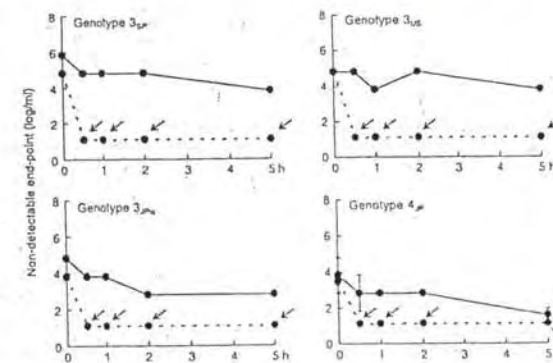


Fig. 1 Inactivation kinetics of the four HEV isolates during liquid-heating. Solid lines: HEV in 25% albumin. Broken lines: HEV in PBS. Arrow: Infectious virus was not detected. Genotype 4_{SP}, n = 3.

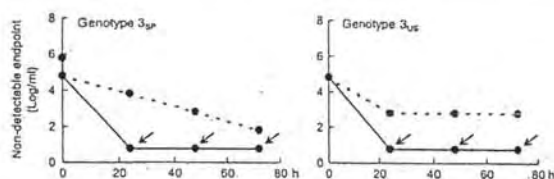


Fig. 2 Inactivation kinetics of the two HEV isolates during dry-heating. Solid lines: at 80°C. Broken lines: at 60°C. Arrow: infectious virus not detected.

Table 2 Viral removal by nanofiltration using filters of various pore sizes

BMM-filtrate	HEV ^a				
	3 _J (swJB-N2)	3 _J (swJB-MS)	3 _J (swJB-E10)	3 _J (cultured HEV ^b)	4 _J (swJB-H1)
BMM-35N (35 ± 2 nm)	[6/1(4/6)] ^c 1.3 ^c	[6/9(3/3)] ≥ 3.6	[6/4(3/8)] 2.6	[6/0(3/2)] ≥ 2.8	[5/6(4/5)] 1.1
BMM-20N (19 ± 2 nm)	[6/1(3/2)] ≥ 3.8	[6/9(3/3)] ≥ 3.6	[6/4(3/2)] ≥ 3.2	[6/0(3/2)] ≥ 2.8	[5/6(3/0)] ≥ 2.6
BMM-15N (15 ± 2 nm)	[6/1(3/2)] ≥ 3.8	[6/9(3/3)] ≥ 3.6	[6/4(3/2)] ≥ 3.2	[6/0(3/2)] ≥ 2.8	[5/6(3/0)] ≥ 2.6

^aHEV is in PBS.

^bGenome amount is indicated as total log copies. Left: before filtration; right: after filtration.

^cLog reduction factor. Log reduction factor was calculated from the genome amount in the samples before and after filtration.

^dDerived from cultured media of HEV-infected A549 cells.

≥ 4.0 after treatment at 80°C for 24 h in any samples. However, although the infectivity of HEV was reduced at an LRF of 2.0 and 3.0, respectively, residual infectivity was detected in all samples that were treated at 60°C for 72 h (Fig. 2). These results indicated that the heat sensitivity is different not by genotype or cluster, but by the composition of the sample.

Filtration of HEV

The putative particle size was also evaluated using Planova filters. All purified HEV isolates were removed to below the detection limit using Planova-15N and -20N, whereas significant amounts of HEV were detected after filtration using Planova-35N. In particular, the removability by Planova-35N was variable for the HEV isolates (Table 2). The result also showed a similar log reduction of viral removal between viruses derived from faeces and cell cultures of genotype 3_J, and suggested that the diameter of viral particles in the purified sample derived from faeces was not affected by contaminants derived from faeces. These results may suggest that the particle size of HEV is around 35 nm, as previously reported [1].

Discussion

Several reports suggested that some industrial swine farms and commercial swine livers in industrial as well as developing

countries could be contaminated by HEV [4,9]. Yazaki *et al.* detected HEV genomes in commercial swine livers that had been eaten by a hepatitis E-infected patient, as shown by the identical sequences of HEV in the liver and patient's sample by genome analysis. They reported that the patient became infected by eating uncooked liver [4]. Our infection studies using piglets demonstrated that HEV was mainly detected in liver, intestines, serum and faeces, but not detected in muscles [17]. Current epidemiological studies revealed that the prevalence of HEV RNA or anti-HEV IgG-positive blood donors in Hokkaido and Tokyo was 0.01% (56/432,167) of RNA and 3.9% of IgG, and 0.01% (3/44,322) of RNA and 8.6% of IgG, respectively. In addition, the prevalence of anti-HEV IgG in Japan varies according to locality, 1.0–8.6% [11]. These results also suggest that although the possibility of transmission is not considered to be high at the moment, some patients who have HEV in their blood may donate blood and this could lead to a transfusion-transmitted infection. Consequently, a monitoring study for donated blood has been initiated in Hokkaido, Japan.

Huang *et al.*, Emerson *et al.*, and Takahashi *et al.* reported on the heat sensitivity of HEV [13–15]. Several strains heated at 56°C for 1 h were sensitive. Some strains were inactivated to below the detection limit whereas in others, < 1% of the virus was still infectious. Unfortunately, these results were not shown with log reduction, time kinetics and effect by stabilizer at 60°C. Furthermore, there has been no report of heat inactivation of freeze-dried samples containing HEV. In

this study, we investigated the heat sensitivity in liquid and dry conditions over longer periods of time using several HEV isolates belonging to genotypes 3 and 4. The results suggest that the inactivation could be greatly influenced by the conditions. In addition, HEV was inactivated gradually at 60°C during dry-heating, whereas it was inactivated to below the detection limit within 24 h at 80°C. This result suggests dry-heating at 80°C to be effective for the inactivation of HEV [18]. The inactivation patterns of HEV at 60°C with albumin and fibrinogen were similar to those of canine parvovirus, which is used as a model of heat-resistant viruses (data not shown). This result suggests that HEV is a heat-resistant virus.

We also evaluated particle size using nanofilters that have a nominal pore size of 15, 19 and 35 nm using isolates from infected swine faeces and from medium cultured with the infected cells. The viral particle size is consistent with a diameter of around 35 nm as reported previously in an electronic microscopic analysis [1].

We reported that the heat sensitivity of parvovirus B19 is also influenced and subsequently varied its inactivation patterns, using different compositions of the inactivation matrix [19]. In addition, although the mechanism of viral particle removal by nanofiltration is size-exclusion, the removal capabilities of these virus-removal filters are also influenced by viral load and the condition/composition of the filtrate [20–23]. Therefore, a safety evaluation for HEV contaminants, especially inactivation by heating and removal using, for example, nanofilters, should be performed using validated manufacturing conditions.

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一般的名称	人全血液	研究報告の公表状況	Piron M, Vergés M, Muñoz J, Casamitjana N, Sanz S, Maymó RM, Hernández JM, Puig L, Portús M, Gascón J, Sauleda S. <i>Transfusion</i> . 2008 Sep; 48(9): 1862-8.	公表国	使用上の注意記載状況・ その他参考事項等
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			スペイン	
研究報告の概要	○カタルーニャ(スペイン)の高リスク供血者における <i>Trypanosoma cruzi</i> 感染の抗体陽性率 背景:ラテンアメリカ人のヨーロッパ(特にスペイン)移入が増加するにつれ、シャーガス病(中南米農村部に流行する人畜共通感染症)などの新たな病原体が認められるようになった。媒介生物サンガメがいない場合、非流行地域のシャーガス病伝播の主な形態のひとつは、輸血を介するものである。 研究デザインおよび方法:カタルーニャ血液銀行は、高リスク供血者におけるシャーガス病スクリーニング計画を実行し、供血者集団で <i>Trypanosoma cruzi</i> (<i>T. cruzi</i>) 感染の血清学的検査陽性率を調査した。全検体にシャーガス抗体試験、ID-PaGIA (DiaMed社)とbioelisaシャーガス検査(Biokit社)の2種類の市販検査を使用した。 結果:全体の血清学的検査陽性率は0.62%であり、検査を実施した高リスク供血者1,770名のうち11名の陽性が確定した。最も陽性率が高かったのはボリビア出身の供血者であった(10.2%)。陽性供血者11名のうち1名は、シャーガス病流行地域に数年間滞在したことのあるスペイン人であった。さらに、陽性供血者の1名は、検出可能な寄生虫血症を呈した。 結論:本試験の結果は、非流行国の高リスク供血者に <i>T. cruzi</i> スクリーニング検査を実施する必要性を強調する。流行地域に居住経験のある(必ずしも当該地域で誕生すること意味するわけではない)高リスクに分類される人々を、検査対象に含めることが重要な知見として挙げられる。 <i>T. cruzi</i> スクリーニングが全供血者に対してルーチンに実施されない場合には、供血前の問診における高リスク供血者の特定が重要である。				人全血液-LR「日赤」 照射人全血液-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			
スペイン、カタルーニャの高リスク供血者集団における <i>T. cruzi</i> 感染の抗体陽性率は0.62パーセントであったとの報告である。		日本赤十字社は、輸血感染症対策として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。日本在住の中南米出身献血者については、厚生労働科学研究「献血血液の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。			

BLOOD DONORS AND BLOOD COLLECTION

Seroprevalence of *Trypanosoma cruzi* infection in at-risk blood donors in Catalonia (Spain)

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BACKGROUND: The increasing arrival of Latin Americans to Europe and, particularly, to Spain has led to the appearance of new pathologies, such as Chagas disease, a zoonotic infection endemic to rural areas of Central and South America. In the absence of the triatomid vector, one of the main modes of transmission of Chagas disease in nonendemic regions is through blood transfusion.

STUDY DESIGN AND METHODS: The Catalan Blood Bank has implemented a screening program for Chagas disease in at-risk blood donors and has performed a study to determine the seroprevalence of *Trypanosoma cruzi* infection in the donor population. The two commercial tests used in all samples were the ID-PaGIA Chagas antibody test (DiaMed) and the bioelisa Chagas assay (Biokit).

RESULTS: Overall seroprevalence was 0.62 percent, with 11 donors confirmed positive among the 1770 at-risk donors studied; the highest rate (10.2%) was in Bolivian donors. Interestingly, 1 of the 11 positive donors was a Spaniard who had resided various years in a Chagas disease endemic area. Furthermore, 1 of the positive donors presented detectable parasitemia.

CONCLUSION: The results of this study emphasize the need for *T. cruzi* screening in at-risk blood donors in nonendemic countries. An important finding is the relevance of including in the at-risk category persons who have resided in, but were not necessarily born in, an endemic region. If *T. cruzi* screening is not routinely performed in all donations, it remains highly dependent on proper identification of at-risk donors during the pre-donation interview.

American trypanosomiasis or Chagas disease is a zoonotic infection endemic to Latin America. In endemic countries, approximately 8 million people are carriers of the disease, approximately 50,000 new cases are diagnosed every year, and fatal cases are estimated at 14,000 per year.¹

Trypanosoma cruzi, the causal agent of Chagas disease, can be detected in blood during the initial acute phase, which lasts from 6 to 8 weeks. Most patients are asymptomatic or oligosymptomatic, but when symptoms manifest, the acute stage of the illness may be characterized by fever, lymphadenopathy, mild splenomegaly, and edema, sometimes involving the myocardial tissue and producing acute myocarditis or encephalomyelitis. If they remain untreated, 5 to 10 percent of these patients die.² After this phase, the infection usually progresses to the chronic stage, in which the parasite is rarely detected in blood. When it is clinically silent, the chronic phase is called the indeterminate form of the disease. Many patients remain in this clinical situation for the rest of their lives, but 15 to 30 percent will progressively develop symptomatic disease.^{2,3} Cardiac manifestations are

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the hallmark of the chronic stage. The most threatening complications are heart failure and excitability and conductivity disorders leading to cardiac arrhythmia and sudden death. These conditions often require recurrent hospitalization, surgery, or more expensive cardiologic procedures such as pacemakers, implantable automatic defibrillators, and even heart transplants.^{2,4} Less frequently, Chagas disease involves the digestive tract.^{2,3}

In endemic areas, Chagas disease is commonly transmitted by a triatomid vector that releases parasite-infected excreta into lacerated skin or mucosa. Congenital and transfusion-related transmission are the other principal modes of acquiring *T. cruzi* infection.^{2,5} Transmission of Chagas disease via blood transfusion has been recognized since 1952,⁶ but it was only with the advent of the HIV pandemic in the 1980s that blood control programs began to be implemented in most Latin American countries. Legislation requiring blood transfusion screening has decreased the incidence of transfusion-related Chagas disease. There are varying degrees of success, however, in implementing these control measures in some endemic regions.⁷

In countries where it is not endemic, such as Spain, Chagas disease is considered an emerging infection because of the increasing number of immigrants coming from Latin America. Spain houses approximately 4 million immigrants, and 1.5 million of them were born in a country endemic for Chagas disease.⁸

Transmission of *T. cruzi* in countries where the vector does not exist occurs mainly through maternal-fetal transmission, organ transplantation, and blood transfusion.⁹ Despite this knowledge and confirmed reports of *T. cruzi* infection through congenital transmission^{10,11} and blood transfusion in nonendemic countries,¹² little attention has been paid to assuring optimal screening and control measures.

Since September 2005, Spanish regulatory law requires that all at-risk donors be screened for Chagas disease or otherwise be excluded from donation.¹³ Donors considered at risk by the Spanish Ministry of Health include persons born in an endemic area, those born of a mother native to an endemic area, and those who have undergone transfusion in an endemic area. The main objective of this article is to estimate the prevalence of *T. cruzi* infection in blood donors in Catalonia through implementation of a *T. cruzi* antibody screening test in donors considered at risk by the Spanish Ministry of Health, as well as all residents for more than 1 month in an endemic area.

MATERIALS AND METHODS

Donor selection and study design

Individuals included in the study belonged to one of the following risk groups: Group 1, donors born or transfused

in an endemic area; Group 2, donors born of a mother native to an endemic area; and Group 3, residents in an endemic area for more than 1 month. For the first group, which was expected to contain the largest number of individuals, we calculated a sample size of 1500 subjects for an estimated prevalence of 0.6 percent of *T. cruzi* infection (95% CI, 0.2%-1%). Blood donation was accepted if there was no other reason for rejection (e.g., malaria). In patients who had grounds for rejection, a blood sample was requested only for *T. cruzi* determination.

Each donor answered an epidemiologic questionnaire to obtain information on age, sex, birth place, date of arrival in Spain, visits to endemic regions in Latin America, and living conditions in the endemic area (rural environment, adobe house). The donors signed an informed consent form and the study design was approved by the Ethics Committee for Research of our center. Clinical assessment and follow-up was offered to all positive donors.

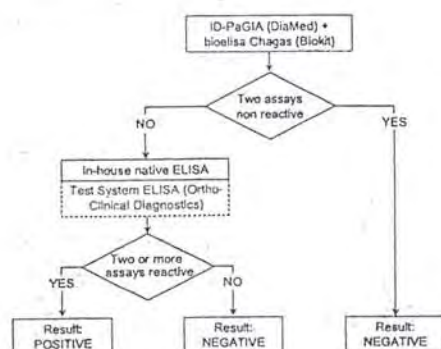
Detection methods

Serum samples from at-risk donors were processed for the presence of *T. cruzi* antibodies by two EC-approved tests, according to the manufacturer's instructions. Each of these tests claimed 100 percent sensitivity based on various performance evaluation studies presented in the insert. Screening was performed with a commercially available Chagas antibody test (ID-PaGIA, DiaMed, Cressier sur Morat, Switzerland), a particle gel immunoassay that contains two recombinant antigens: Ag2 and TcE. All blood donations with an initially reactive result in the screening test were rejected. It should be noted that independently of the result of Chagas determination, platelet concentrates were not made from at-risk donors.

The second test used in all samples was the Chagas bioelisa assay (Biokit, Llíçà d'Amunt, Spain), which also contains a recombinant antigen, TcF antigen (*T. cruzi* fusion protein), and consists of a linear assembly of four serologically active peptides PEP-II, TcD, TcE, and TcLoE1.2. When a positive result was obtained in at least one of these tests, a conventional in-house enzyme-linked immunosorbent assay (ELISA) test utilizing whole *T. cruzi* antigens from Maracay strain epimastigotes was also performed. Samples were confirmed positive when at least two tests gave a positive result (Fig. 1).

All initially positive samples by ID-PaGIA Chagas antibody test and/or Chagas bioelisa assay were retrospectively tested with the *T. cruzi* ELISA test system (Ortho-Clinical Diagnostics, Raritan, NJ), which was FDA- and EC-approved after the beginning of this study. This last test uses epimastigote lysate antigens.

Furthermore, all initially positive samples were assessed for the presence of parasite DNA in blood, using in-house real-time polymerase chain reaction (PCR).¹⁴

Fig. 1. Algorithm for *T. cruzi* serology interpretation.

The PCR technique is designed to amplify a highly represented fragment of 166 bp in the satellite DNA of *T. cruzi*, it contains an internal control for DNA extraction and amplification (human RNase P gene), and has an estimated sensitivity of 2 parasites per mL (95% positive hit rate).

RESULTS

Epidemiologic data

Between September 2005 and September 2006, a total of 1770 donors were enrolled in the prevalence study and were screened for *T. cruzi* antibodies. These individuals accounted for 1.1 percent of all blood donors in the first 3 months of the study (Table 1).

Sex distribution (51% men) was similar to that of the general Catalanian donor population (53% men), whereas the mean age was lower than that of the general donor population (35 ± 11 years vs. 42 ± 12 years). Approximately half the donors included in the study arrived to Spain after 2000, 5 years before the beginning of recruitment for the study.

According to risk groups, 1524 (86.1%) individuals were born in an endemic area (Group 1), 37 (2.1%) were born of a mother from an endemic area (Group 2), and 209 (11.8%) were temporary residents in an endemic country (Group 3; Table 1). Twenty-one donors (1.2%) stated that they had undergone transfusion in a country endemic for Chagas disease. Only 20.7 percent of donors born in an endemic area stated that they had lived in a rural environment and only 9 percent declared to have lived in an adobe house. For temporary residents, the proportions were 66.5 and 22 percent, respectively (Table 2).

The most highly represented country of origin was Colombia, accounting for 22.3 percent of at-risk donors included in the study, followed by Argentina and Ecuador, accounting for 19.5 and 14.6 percent, respectively

(Table 3). The majority of mothers of the 37 donors in Group 2 came from Argentina (10), followed by Colombia (7), Chile (7), and Peru (3). Most donors from Group 3 (n = 209) had visited various endemic countries during one or several trips.

Prevalence of *T. cruzi* infection in blood donors in Catalonia

In the serologic screening, 21 donors presented an initially reactive result by ID-PaGIA Chagas and 25 by bioelisa Chagas. Samples showing faint agglutination with the use of ID-PaGIA or an inconclusive result with bioelisa (ratio absorbance/cutoff between 0.9 and 1) were considered initially reactive. Only 11 donors were reactive in both tests. The third test (in-house ELISA) was only positive in the 11 serum samples that resulted positive by the two commercial tests used in the screening (Table 4). The results obtained with the *T. cruzi* ELISA test system (Ortho-Clinical Diagnostics) agreed with those obtained with the in-house ELISA (35/35), also based on whole parasite lysate antigens. In addition, 1 of the 11 donors had detectable parasitemia by PCR analysis.

Overall prevalence was 0.62 percent in the at-risk population. Ten of the eleven positive donors were from Group 1 (0.66%), and one was from Group 3 (0.48%) (Table 5). The countries of origin of positive donors were Bolivia (6 cases), Argentina (2), Ecuador (1), and Paraguay (1), and there was one Spaniard who had been living in Venezuela for 27 years. We should emphasize that the number of positive subjects among Bolivians (6 out of 59 Bolivian donors) represents a prevalence of 10.2 percent for this country. None of the 37 donors born of a mother native to an endemic area and none of the donors transfused in an endemic area (n = 21) were positive for *T. cruzi* antibodies. Only 3 of the 11 positive donors declared that they had been living in a rural area or an adobe house (Table 5).

DISCUSSION

In endemic countries, blood transfusion is the second most important way to acquire Chagas disease. Screening coverage in blood banks has reached 100 percent in many countries, and this has reduced the risk of transmitting the infection by transfusion.¹⁵ Nevertheless, cases of *T. cruzi* transmission by blood transfusion have been recently described in Mexico where screening coverage, which is not mandatory at this time, is one of the lowest of all Chagas disease endemic countries.^{15,16}

In nonendemic countries, blood transfusion is one of the main modes of acquiring the infection, and cases of transmission before screening for *T. cruzi* infection became mandatory in blood donors have been reported in Spain.^{17,18} European legislation requires permanent rejection

TABLE 1. Epidemiologic data of donors included in the study

Donors included by group of risk	Number (%)	Transfused in endemic area*	Sex		Deferred before donation*	Age (years)†
			Male*	Female*		
Group						
1. Born in an endemic area	1524 (86.1)	21 (1.4)	758 (49.7)	766 (50.3)	95 (6.2)	35 (10.7)
2. Born of a mother native to an endemic area	37 (2.1)	0	18 (48.6)	19 (51.4)	1 (2.7)	28 (10.0)
3. Temporary resident in an endemic area	209 (11.8)	0	119 (56.9)	90 (43.1)	19 (9.0)	38 (10.7)
Total	1770	21 (1.2)	895 (50.6)	875 (49.4)	115 (6.5)	35 (10.8)

* Data are reported as number (%).

† Data are reported as mean (SD).

TABLE 2. Living conditions in endemic area

Group 1: donors born in endemic region	Group 3: resident in endemic region	
	Has lived in adobe house	Has lived in adobe house
Has lived in rural area		
315/1524 (20.7%)	137/1524 (9.0%)	139/209 (66.5%)
		46/209 (22.0%)

TABLE 3. Distribution of donors born in an endemic region and of positive donors by country of origin

Country	Tested for anti- <i>T. cruzi</i> *	Percentage of official immigrant population in Catalonia	Number	Anti- <i>T. cruzi</i> -positive donors	
				Rate by country (%)	
Colombia	340 (22.3)	13.8			
Argentina	298 (19.5)	11.7	2	2/298 (0.67)	
Ecuador	223 (14.6)	29.2	1	1/223 (0.45)	
Uruguay	127 (8.3)	4.4			
Peru	123 (8.1)	8.9			
Brazil	113 (7.4)	3.9			
Venezuela	86 (5.6)	2.4			
Chile	77 (5.0)	4.2			
Bolivia	59 (3.9)	8	6	6/59 (10.2)	
Mexico	40 (2.6)	2.6			
Paraguay	15 (1.0)	1.1	1	1/15 (6.7)	
Honduras	10 (0.7)	1.3			
El Salvador	6 (0.4)	0.4			
Nicaragua	3 (0.2)	0.1			
Costa Rica	2 (0.1)	0.1			
Guatemala	1 (<0.1)	0.1			
Panama	1 (<0.1)	0.1			
Total	1524		10		

* Data are reported as number (%).

tion of persons with a history of Chagas disease for blood donation.¹⁹ Nevertheless, most people do not present any health problem until many years after acquiring the infection. Because of the increasing number of people from Latin America residing in Europe, and European people who reside for a time in an endemic area, implementation of screening programs for this disease in at-risk donors may be advisable in all European blood banks.

The Catalanian Blood Bank implemented a screening program for Chagas disease in all at-risk donors and simultaneously initiated a study to determine the seroprevalence of *T. cruzi* infection in its blood donor population. The countries of origin of the largest percentages of at-risk donors in the present study were Colombia,

TABLE 4. Distribution of results obtained with the two commercial kits ID-PaGIA (DiaMed) and bioelisa Chagas (BioKit)*

Initial result with ID-PaGIA	Initial result with the bioelisa Chagas	
	Positive	Negative
Positive	11†	10‡
Negative	14†	1735

* All initially reactive results were confirmed as positive or negative by in-house native ELISA. Cohen's kappa index, 0.471.²¹

† In-house native ELISA result positive.

‡ In-house native ELISA result negative.

TABLE 5. Epidemiologic data of the 11 positive donors

Positive donor number	Sex (male/female)	Age at donation (years)	Country	Town, State	Did you live in a rural area?	Did you live in an adobe house?	Born in Spain	Date of arrival in Spain	Have you returned recently to your country?	Transfusion in an endemic country
1	F	34	Ecuador	Machala, El Oro	No	No	No	2000	Yes	No
2	F	34	Bolivia	Cochabamba, San Benito	Yes	Yes	No	2002	No	No
3	F	42	Argentina	Guaymas, Mendoza	Yes	Yes	No	2002	No	No
4	F	36	Bolivia	Santa Cruz, Santa Cruz	No	No	No	2005	Yes	No
5	M	38	Bolivia	Santa Cruz, Santa Cruz	No	No	No	2004	No	No
6	M	45	Bolivia	Santa Cruz, Santa Cruz	No	No	No	2003	No	No
7	F	31	Venezuela	Caracas	Yes	Yes	Yes	2003	No	No
8	F	36	Bolivia	Cochabamba, Cochabamba	No	No	No	2003	No	No
9	F	40	Bolivia	Santa Cruz	No	No	No	2003	No	No
10	F	49	Argentina	San Juan	No	Yes	No	1988	No	No
11	F	51	Paraguay	San Esteban, San Pedro	No	No	No	1978	Yes	No

Argentina, and Ecuador, and these were also the countries of origin of the largest percentages of immigrants in Catalonia in 2005 (Table 3).⁸

Overall seroprevalence was 0.62 percent in the 1770 at-risk donors included, and positive donors were mainly from Bolivia, with a 10.2 percent prevalence among donors from this country. The seroprevalence of *T. cruzi* infection in Bolivian donors is very high and is in keeping with the 9.9 percent reported in 2001 in that country (86.1% screening coverage at the time of the study), which is the most highly affected by Chagas disease.¹⁵ The remaining positive donors born in endemic areas were from Argentina, Paraguay, and Ecuador. The seroprevalence of *T. cruzi* infection in blood donors reported in 2001 or 2002 for these countries was 4.5 percent (second most highly affected country), 2.8 percent (third most highly affected country), and 0.4 percent, respectively.¹⁵

One important finding of this study is the relevance of including persons who have resided in, but were not necessarily born in, an endemic area as an at-risk donor group for *T. cruzi* infection. This population is not considered at risk in the current Spanish regulations.¹³ One of the 11 positive donors described herein was born in Spain and had resided for many years in Venezuela.

Various studies have reported seroprevalence data in the immigrant population and in blood donors in countries that are not endemic for Chagas disease. In Canada and Germany, for example, seroprevalences of 1 and 2 percent have been described, respectively, in cohorts of asymptomatic immigrants coming from Latin America.^{20,21}

As to blood donors, two recent surveys in the United States reported a seroprevalence of 0.02 to 0.03 percent among all donors in blood centers in California, Arizona,²² and Texas.²³ A previous study carried out in Los Angeles and Miami blood centers identified 7.3 and 14.3 percent of donors as at risk for Chagas disease, with a 0.2 and 0.1 percent seroprevalence of *T. cruzi* infection, respectively, in these at-risk populations.²⁴

In Spain, some blood banks have implemented Chagas' disease screening in at-risk donors and seroprevalence data have been described, although some of the results are preliminary. *T. cruzi* infection seroprevalence varies from 0.05 to 1.38 percent in the available studies.^{17,25-27} A mean seroprevalence of 0.65 percent can be calculated from data proceeding from all Spanish blood centers that have performed (or initiated) a survey, including, as a whole, 10,388 blood donors at risk for *T. cruzi* infection. The results obtained in Catalonia are consistent with these data.

The epidemiologic questionnaire provided some interesting information. First, the mean age of the at-risk donors proceeding from an endemic area (Group 1 donors) is lower than the general no-risk population (35 years vs. 42 years), as would be expected in immigrants who generally come to Spain to work and improve their

living conditions. Half the population included arrived in Spain after 2000, a fact that illustrates the increasing immigration rates from Latin America observed over the past years. Another interesting result from the questionnaire was that the information obtained about living conditions in the Chagas disease endemic area (rural area, adobe house) did not correlate with the presence or absence of antibodies to *T. cruzi*. People born in endemic regions (7 of 11 positive donors) generally declared that they had never lived in a rural environment or an adobe house (Table 2), as is commonly assumed. Hence, this question is not useful for differentiation purposes. Interestingly, the same conclusion was drawn from the Berlin study, in which 95 of 100 immigrants declared that they came from an urban area, including the 5 cases of confirmed Chagas disease.²¹

The two serologic assays used in this study were chosen because at the beginning of the study they were commercially available and EC-marketed. Both are based on recombinant antigens, whereas the third conventional in-house ELISA is based on whole parasite lysate. All samples confirmed as positive had been initially reactive with both recombinant antigens assays, and all samples initially reactive with only one assay presented a nonreactive result in the in-house ELISA and were considered false-positive samples. It is worth noting that many discrepant results observed between both assays corresponded to low 0.9 to 1 signal-to-cutoff rates for bioelisa Chagas (Blok) or doubtful reactions with ID-PaGIA (Diamed), which were all considered as initially reactive in this study. Additionally, it should be mentioned that the *T. cruzi* ELISA test system performed on all initially reactive samples (with one or two tests) confirmed the results obtained with the conventional in-house ELISA. The high rate of inconclusive or false-positive results obtained when one diagnostic test is used underscores the need to confirm all initially positive results with a second serologic technique. In any case, there is still a need for a real confirmatory test to overcome the issues of discrepancies and false results (positive or negative). The ID-PaGIA assay allows testing of a small number of samples at a time. Although this system has the drawback of rather subjective reading, it could be useful in blood centers with a small volume of donations and is now even more reliable since a third antigen has been recently added to increase the sensitivity of the test. The ELISA format, which allows for automation and objective reading, should be indicated in other blood centers. An even more appropriate strategy would be the use of two screening tests, one based on recombinant antigens and the other on crude antigens.²⁸

In summary, this study reports a seroprevalence of *T. cruzi* infection of 0.62 percent among at-risk donors in Catalonia and emphasizes the need to include individuals who have resided in, but were not necessarily born in

endemic areas as at-risk donors. The difficulty of this type of selective screening is proper identification of the risk population, which essentially depends on the predonation interview. Latin Americans accounted for more than 1 percent of the total of donors in our study, and this substantial contribution underscores the need to accept them as donors.

In the future, techniques to inactivate or reduce the parasite load, which are currently under development or evaluation,^{29,30} might be applicable to blood components. At this time, however, detection of *T. cruzi* infection is the only preventive measure available to accept at-risk blood donors.

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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 2008. 10. 15	新医薬品等の区分 該当なし	総合機構処理欄	
一般的名称	人全血液	研究報告の公表状況	BuaNews online, Mon 13 Oct 2008. available at http://www.buanews.gov.za/news/08/081013111151006	公表国	南アフリカ	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)					
研究報告の概要	○アレナウイルスと特定された未知の疾患 南アフリカ、ヨハネスブルグで3名の死者を出したウイルスは、暫定的に西アフリカのラッサウイルスに近い、齧歯類媒介性アレナウイルスであると特定された。ウイルスは感染マウスの排泄物を介し、人間の食物やハウスダストを汚染する可能性がある。南アフリカ国立感染症研究所(NICD)と保健省は共同で、このウイルスが体液を介してヒトからヒトに感染するため、「患者の看護に特別な予防的措置が必要である」との声明を発表した。3名の死因を確定するには更なる検査が必要である。新たなアレナウイルスであるかどうか、ならびに当該ウイルスの分布について検討を行う必要がある。ヒトに疾患を引き起こすアレナウイルスが南アフリカの齧歯類に存在することはまだ示されていないとNICDは述べた。 1人目の女性患者は、9月中旬に重篤な容態でザンベアから搬送され、Morningside Medi-Clinicに入院し、2日後に死亡した。約2週間後、1人目の患者の搬送に同行した救急救命士が死亡し、間もなく看護師が死亡した。 1人目の患者と接触した他の3名の患者は退院したことが確認されているが、依然として2名が厳重な監視下に置かれている。1名は、発熱およびインフルエンザ様症状を発症した救急救命士であり、もう1名は2人目の患者をケアした女性看護師である。彼女は、隔離され抗ウイルス剤ribavirinの投与を受けており、現在は安定している。				使用上の注意記載状況・その他参考事項等 人全血液-LR「日赤」 照射人全血液-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				
南アフリカ、ヨハネスブルグで3名の死者を出したウイルスは、暫定的に西アフリカのラッサウイルスに近い、齧歯類媒介性アレナウイルスであると特定されたとの報告である。		日本赤十字社では、輸血感染症対策として問診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。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。				