資料2

感染症定期報告に関する今後の対応について

平成１６年度第５回
運営委員会確認事項
（平成１６年９月１７日）

1 基本的な方針

運営委員会に報告する資料においては、

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

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

2 具体的な方法

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

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

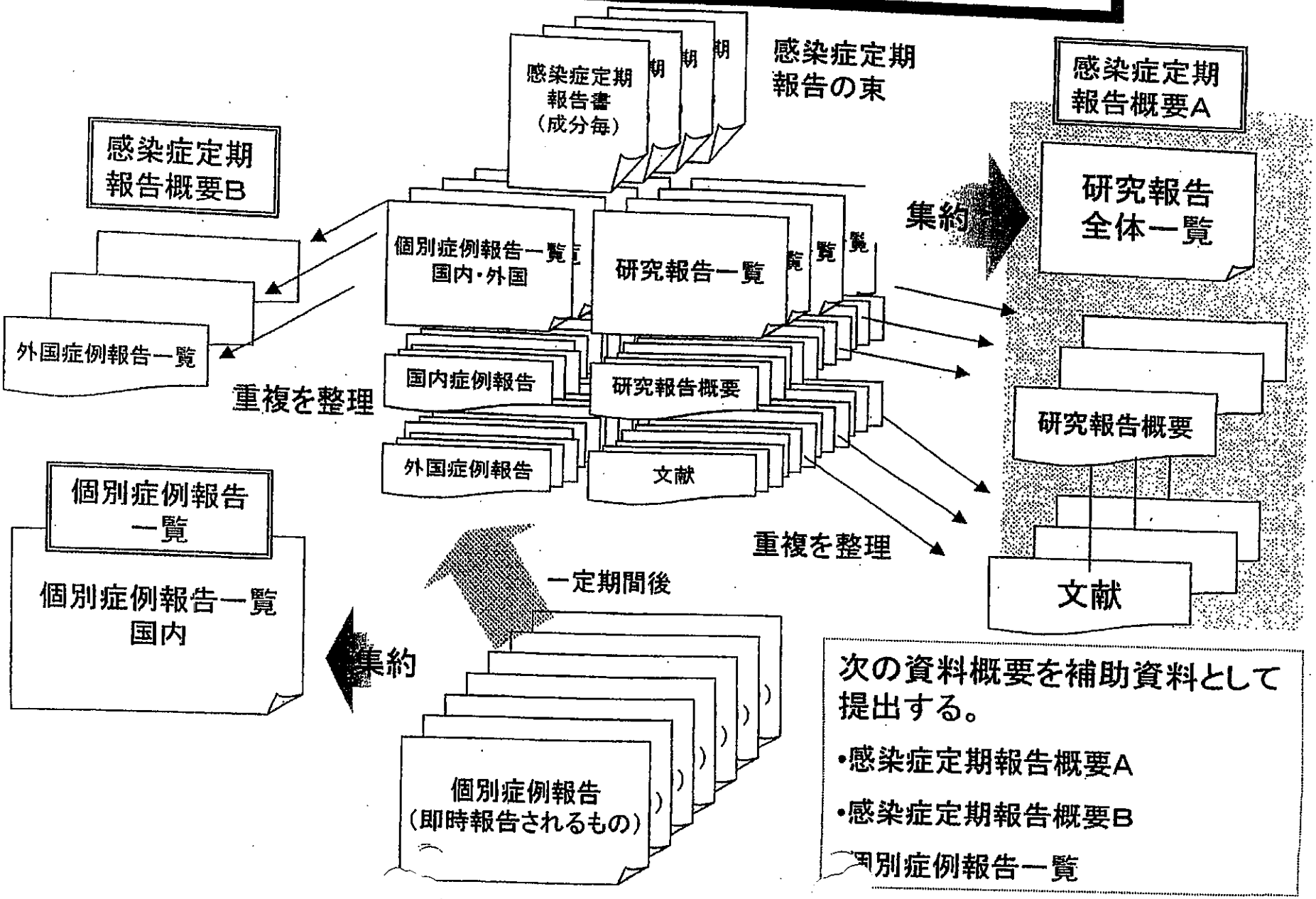
② 感染症発生症例報告のうち、発現国が「外国」の血漿分画製剤の使用による症例は、同一製品毎に報告期間を代表する感染症発生症例一覧（別紙様式第4）をまとめた「**資料概要B**」を事務局が作成し、送付する。

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

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

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

感染症定期報告・感染症個別症例報告の取り扱い



感染症定期報告概要

（平成２４年３月１４日）

平成２３年１０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２３年１０月１日以降に報告された感染症定期報告に含まれる研究報告（論文等）について、重複している分を除いた報告概要一覧表を作成した。

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

感染症定期報告の報告状況（2011/10/1～2012/1/31）

血対ID	受理日	番号	感染症（PT）	出典	概要	新出文献番号
100466	14-Dec-11	110740	B型肝炎	J Clin Virol. 52(2011)151-154	B型肝炎ウイルス（HBV）ワクチンが無効であった慢性B型肝炎感染の症例報告。2006年4月、同性間性交渉を持つ男性に対してHBVワクチン接種が実施され、ワクチンの増量の後2007年11 月には抗HBs抗体が2社の検査法で161mIU/mL及び62mIU/mLになった。2009年12月、患者が疲労感と筋肉痛のため受診したところ、ALT上昇が見られ、検査により慢性HBV感染症であることが確認された。系統発生解析によりこのウイルスはHBVジェノタイプFに一致した。これは、HBVワクチンを接種され抗HBs抗体価が10mIU/mLを上回った者が、HBVジェノタイプFに感染し、慢性化した初めての報告である。	1
100494	30-Jan-12	110879	B型肝炎	http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/default.htm	米国FDAによる、血液製剤のB型肝炎ウイルス（HBV）」感染リスクを低減させるための核酸検査に関するガイダンス案。血液事業者に対し主に以下の事項が勧告されている。 ・人全血液及び血液成分については、HBsAg及びHBcAb陰性の検体に対しHBV NATを実施し、検出限界は100 IU/mLとする。原料血漿についてはHBsAgが陰性の検体に対しHBV NATを実施し、検出限界は500 IU/mLとする。 ・他の感染症関連検査がすべて陰性であれば、輸血用人全血液、血液成分及び原料白血球については、HBV NAT、HBsAg、HBcAbが陰性であること、原料血漿及び回収血漿についてはHBV NAT及びHBsAgが陰性であることをもって、供給を可とする。 ・.NAT結果が陽性の場合、自己血を除いて、供給してはならない。永久供血延期または無期限供血延期とする。 ・供血者の適格性再確認には、少なくとも陽性となった採血から6カ月後にHBsAg、HBcAb及びHBV NATを行い、その結果により、永久的供血延期かリエントリー可能か評価する。	2
100434	24-Oct-11	110642	B型肝炎 C型肝炎	Interviorology. 54(2011)185-195	日本におけるC型肝炎ウイルス（HCV）、B型肝炎ウイルス（HBV）の診断未確定キャリア総数に関する報告。地域及び年齢別のHCVならびにHBV有病率を、20-39歳については初回供血者、40-74歳については定期健康診断の受診者にて調査された。5-19歳の若者の有病率は一つの県において決定され、75歳以上の高齢者のHCV有病率は指数モデルにて推定された。その結果、2005年の127,285,653人の全人口のうち、0.63%がHCVキャリアであると算出され、0.71%がHBVキャリアであると算出された。C型肝炎の年齢による特徴として、年齢とともに有病率の変動幅が大きかった。地域別の特徴として、有病率の変動幅が大きく異なり、西日本で増幅が大きい傾向があった。一方B型肝炎では、全地域において55～59歳で最も有病率が高く、北海道では3.1%に上った。どの地域においても2000年と2005年を比較すると、HCV及びHBVキャリアの割合は減少している。	3
100432	24-Dec-11	110639	E型肝炎	Vox Sanguinis. (2011)1-2	血漿分画プールにおけるE型肝炎ウイルス（HEV)の地理的分布に関する報告。欧州、北米、中東及びアジアから血漿分画プール75例を入手し、HEV RNAと抗HEV IgGの検出を行った。その結果、約10%の血漿分画プールがHEV RNA陽性で、陽性プールの地理的分布は欧州、北米、アジアと広範囲にわたっていたが、いずれも1000copies/mlを超えていなかった。同定された株の系統発生解析により、genotype4がアジアのプールに限定されていたのに対し、genotype3は欧州と北米のプールで確認された。また、抗HEV IgGの濃度はアジアのプールで最も高かった。	4
100472	26-Dec-11	110773	E型肝炎	Vox Sanguinis. Sep 29, 2011	英国における献血者プール血漿中のE型肝炎ウイルス（HEV）RNAの検出に関する報告。血液供給へのHEV感染リスクを調査するために、英国において収集された880例の血漿ミニプールを対象に血清学的及び分子学的調査が行われた。それぞれのプールは48人分の献血から構成されていた。検査の結果、6例（0.7%）のプールにおいてHEV-RNA陽性であり、この6例は全て抗HEV抗体陽性であった。また、HEV-RNA陰性プールのうち100例を検査したところ、73%がHEV IgG陽性であったが、HEV IgM陽性のプールはなかった。これらの結果は血液製剤のHEV感染リスクの可能性を示したものだが、輸血後のHEV感染の範囲については十分な調査がなされていないため、HEVと血液製剤の安全性については更なる調査が必要である。	5

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100472	26-Dec-11	110773	パルボウイルス	Transfusion. 51(2011)1896-1908	パルボウイルスB19(B1V9)の血液分画における分布及び持続性に関する報告。パルボウイルスに対するレセプターが赤血球膜上にあることを踏まえて、B19Vの血中分布を調査するため、B19Vをスパイクした血液及びB19V感染ドナーから収集された血液を用いてウイルスDNAの血液分画中の分布を調査した。ウイルススパイク実験では、血液を超遠心分離法によって分画とし、PCRによりウイルスDNAを定量したところ、DNAの約3分の1は血漿中で回収され、3分の2は赤血球に結合していた。また、血中B19V-DNA濃度が100IU/mL以上でIgM陽性期の感染ドナーにおいて、全血と血漿中のウイルスDNA量を比較したところ、DNA濃度の中央値は血漿中よりも全血中で約30倍高かった。一方で、血中のウイルス濃度が低く、IgM陰性時のドナーでは、全血対血漿比は約1であった。これらの結果より、血漿に対する全血のB19V-DNAの比は、ウイルス量低下とIgM反応性低下を伴って減少することが明らかとなった。	6
100472	26-Dec-11	110773	パルボウイルス	Vox Sanguinis. Jul 22, 2011	パルボウイルスB19(B19V)の液状加熱における熱感受性に関する報告。B19V遺伝子型2の熱感受性を調査するために、アルブミン、免疫グロブリン、ハプトグロビン、アンチトロンビンの製造における熱処理工程の直前に採取した検体にB19Vをスパイクし、60℃10時間の加熱処理を行いながら感染性を経時的に測定した。また、低pH免疫グロブリン溶液についてもB19Vをスパイクし、室温で14日間の処理を行った。その結果、B19V遺伝子型2はアルブミン及び免疫グロブリンにおいて急速に不活性化され、ハプトグロビンにおいては速度は遅いものの不活性化が確認された。一方でアンチトロンビンにおいては不活性化が限定的であり、10時間の加熱処理では感染性が残存していた。また、低pH免疫グロブリン溶液においては7日後に不活性化が確認され、これらの結果は全て遺伝子型1でのパターンと同様であった。このことから、B19Vの遺伝子型1及び遺伝子型2は、異なる血漿製剤の間で熱感受性が変化することが示された。	7
100472	26-Dec-11	110773	パルボウイルス	Journal of Virological Methods. 178(2011)39-43	パルボウイルスB19(B19V)に対するフィルター処理のウイルス除去能に関する報告。15～19nmのフィルターのウイルス除去能力を調査するために、アンチトロンビン、ハプトグロビン、免疫グロブリンのそれぞれの製剤にB19Vを添加し、フィルター処理後に感染力分析とquantitative定量的(Q)-PCR分析を行った。その結果、全ての検体において、フィルター処理後の検体は感染力が示されなかったが、ウイルスDNAはQ-PCRにより検出可能であった。しかし、15nmフィルター濾過後の溶液においてウイルスゲノムのサイズは約90%が0.5kb未満であることが確認された。この結果より、フィルター処理によるリダクションファクターは遊離のDNAにより過少評価されている可能性が示唆された。	8
100428	03-Oct-11	110556	インフルエンザ	MMWR. 60(2011)1213-1215	アメリカにおけるインフルエンザウイルスの再集合に関する報告。2011年8月、米国においてブタインフルエンザ(H3N2)感染症例が2例報告された。2症例の間に疫学的関連性は特定されていない。2症例のウイルスは過去に特定されたH3N2ウイルスと類似しているが、8つの遺伝子のうち1つ(M遺伝子)が2009年のインフルエンザA(H1N1)ウイルスに由来するものであった。このことから、2症例に感染したウイルスがブタインフルエンザA(H3N2)ウイルスとインフルエンザ(H1N1)ウイルスの再集合体であることが示唆された。	9
100459	28-Nov-11	110717	インフルエンザ	Transfusion. 51(2011)1949-1956	日本における、輸血によるインフルエンザ(パンデミック[H1N1]2009)感染リスクに関する報告。日本赤十字社血液センターでは献血後情報としてパンデミック(H1N1)2009感染疑いのある献血者から得られた血液製剤の供給を中止した。輸血による感染リスクを調査するため、献血後7日以内にパンデミック(H1N1)2009と診断された579人の献血者から得られた計565の血漿製剤と413の赤血球製剤についてリアルタイムRT-PCRを実施したところ、どのサンプルからもウイルスRNAは検出されなかった。輸血によるパンデミック(H1N1)2009の感染リスクは極めて低いと考えられる。	10

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100484	23-Jan-12	110811	レトロウイルス(XMRV)	Sciencexpress. 334(2011)814-817	Blood XMRV Scientific Research Working Group (SRWG)による、異種指向性マウス白血病ウイルス関連ウイルス(XMRV)を含むマウス白血病ウイルス(MLV)と慢性疲労症候群(CFS)に関する報告。以前にXMRV/MLV陽性と報告された被験者15例(うち14例がCFS患者)及び以前にXMRV/MLV陰性と報告された健康ドナー15例から採取した血液検体を用いて、再度XMRV/MLV-DNAの検出、ウイルス複製能及び抗体検出の検査を行った。サンプルをコード化し二重盲検下で9か所の研究所に分配し、検査を行ったところ、2か所の研究所においてXMRV/MLV陽性と判定されたサンプルがあったが、CFS患者とコントロールとの間で陽性率に差はなかった。今回の試験により、 XMRV/MLV検査に再現性は確認できず、供血者へのスクリーニング項目として採用する正当な理由はないことが示唆された。	11
100466	14-Dec-11	110740	ニパウイルス	Epidemiol Infect. 138(2010)1630-1636	バングラディシュにおけるニパウイルス(NiV)感染の報告。NiVのヒト-ヒト感染について検討するために、バングラデシュにてNiVに感染した患者1例とその患者と身体的接触のあった知人14例の動向を調査した。その結果、14例のうちNiV感染を発症したのは6例(43%)であった。未感染群と比較して感染群ではNiV患者の咳嗽中に同室に滞在していた割合が高かった。NiVの感染拡大を防ぐためには、患者との身体的接触を最小限とすることが求められる。	12
100432	24-Oct-11	110639	ウイルス感染	Plos Pathogens 7(7); e1002155; 2011	異種間で伝藩する新規アデノウイルスに関する報告。カルフォルニア国立霊長類研究所(CNPRC)において、サル(titi monkey)にアウトブレイクを起こした新規アデノウイルス(TMAdV)が特定された。建屋内のサル65例のうち23例が劇症の肺炎に進行する上気道症状と肝炎を発症し、そのうち19例が死亡又は安楽死とした。集団発生時にサルと接触した研究者も急性呼吸器疾患を発症し、回復期に血清中TMAdVが陽性であることが確認された。また、米国西部におけるランダムな成人供血者81例のスクリーニングにおいて2例にTMAdV特異的中和抗体が検出された。TMAdVの発見は、新規アデノウイルスが異種間アウトブレイクの潜在的原因として厳重に監視される必要があることを示している。	13
100442	28-Oct-11	110661	ウイルス感染	CCDR Weekly Infectious Diseases News Brief. July 22,2011	異種間で伝藩する新規アデノウイルスに関する報告。カルフォルニア国立霊長類研究所(CNPRC)においてサル(titi monkey)にアウトブレイクを起こした新規アデノウイルス(TMAdV)について、集団発生時にサルと接触した研究者も急性呼吸器疾患を発症した。また、研究者の家族にも同様の症状が2例に認められ、血中TMAdVに対する抗体が陽性であることが確認された。研究者の家族は感染サルへの接触がないことから、このウイルスがヒト間でも感染を引き起こした可能性が示唆された。一方、ヒトからサルに感染した可能性も否定できないと報告されている。	14
100459	28-Nov-11	110717	ウイルス感染	Emerging Infectious Diseases. 17(2011)1417-1420	米国における新種のアレナウイルス感染の報告。米国で急性中枢神経疾患や鑑別不能型熱性疾患の患者1,185例中41例(3.5%)から抗ホワイトウォーターアロヨウイルス(WWAV)抗体又は抗リンパ球性脈絡髄膜炎ウイルス(LCMV)抗体が検出された。ペア血清サンプルの抗体価の分析結果から、ノースアメリカンタカリベセロコンプレックスウイルス(NATSV)が2例、LCMVが3例の疾患原因であると示唆された。この研究結果より、NATSVもLCMVと同様に米国内でヒトの疾患原因となることが明らかとなった。	15
100434	24-Oct-11	110642	セラチア	http://www.adph.org/news/assets/110407.pdf	米国における完全静脈栄養剤(TPN)によるSerratia marcescens (S.marcescens)感染の報告。アラバマ州公衆保健局(ADPH)は2つの病院から、TPNを投与した患者にS.marcescens感染が生じたという報告を受けた。同一業者からTPNを納入していた6つの病院が特定され、19症例が報告された。遺伝子解析の結果、TPNを製造する際に使用していた器具及びTPNから分離された菌と、TPNを受けた入院患者12人から分離されたS.marcescensが同じであったと確認した。さらにTPNの原料である混合アミノ酸1袋も、S.marcescensで汚染されていた。TPNを混合する時の殺菌工程の失敗が、汚染の原因になったと考えられる。この製造業者は通知を受け、汚染の可能性を情報提供し、生産を中止し、製品を回収した。	16

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
100434	24-Oct-11	110642	大腸菌性胃腸炎	Eurosurveillance vo.16 Is.24	志賀毒素／ベロ毒素産生大腸菌(STEC／VTEC)感染のアウトブレイク発生時におけるドイツのサーベイランスに関する報告。2011年5月、ドイツのロベルト・コッホ研究所は、STEC／VTEC感染による溶血性尿毒症症候群(HUS)患者の急激な増加を受け、サーベイランスについて以下のような変更を行った。疫学情報交換の集約化／国レベルまでの情報伝達の迅速化／病院の救急部における出血性下痢症の症候群サーベイランスシステムの導入／ドイツにおけるHUS治療受け入れ能力の評価／検査機関でのアクティブサーベイランスの開始。これらの追加サーベイランスシステムは今回のアウトブレイクにおいて、より迅速なモニタリングを可能にし、患者の発生動向等について把握することができた。	17
100432	24-Oct-11	110639	細菌感染	CDC/MMWR. 60(2011)1083-1086	米国におけるレジオネラ症発生状況に関する報告。2000～2009年に国立届出疾病監視システム(NNDSS)に報告されたレジオネラ症(レジオネラ病及びポンティアック熱)症例を評価したところ、米国のレジオネラ症年間報告数は2000年の1,100例から2009年の3,522例へと増加し、粗国内発生率も2000年0.39/10万人から2009年1.15/10万人と増加傾向が確認された。	18
100432	24-Oct-11	110639	細菌感染	N Engl J Med. 365(2011)422-429	米国における新規エーリキア症に関する報告。米国におけるエーリキア症の原因菌について調査するために、患者の血液検体について分子学的方法、培養及び血清学的検査を行った。その結果、ミネソタ州とウィスコンシン州の4症例について、既に知られているE. chaffeensis 、E. ewingiiではない新規のエーリキア種により引き起こされたことが明らかとなった。全ての患者は発熱、倦怠感、頭痛及びリンパ球減少症がみられ、3例は血小板減少症、2例は肝酵素濃度上昇を有していた。全例がドキシサイクリン治療により回復した。また、ミネソタ州とウィスコンシン州で採取された697例のクロアシマダニの少なくとも17例は、同一のエーリキア種がPCR検査陽性であった。遺伝子解析により、この新規エーリキア種がE. murisと近縁種であるしていることが示された。	19
100434	24-Oct-11	110642	細菌感染	21st Regional Congress of the International society of Blood Transfusion; P-384 June18-22,2011	赤血球輸血によるヒト顆粒球アナプラズマ症(HGA)感染に関する報告。36歳女性が帝王切開術を受け、6単位の赤血球と2単位の新鮮凍結血漿が輸血された。9日後に発熱を生じ、後に急性呼吸窮迫症候群(ARDS)に転帰した。PCRによってアナプラズマ・ファゴサイトフィルムが検出され、HGAであることが確認された。感染原因として可能性のあるものは輸血のみであった。輸血された8単位についてPCR及び間接蛍光抗体法により検査を行った結果、1検体が陽性を示した。白血球除去赤血球の輸血により感染が発生したため、白血球除去の効果は小さい可能性が示された。この感染症例は、ダニ咬傷歴のある供血者の一時的な供血停止を支持している。	20
100459	28-Nov-11	110717	感染	IASR. 32(2011)218-219	日本におけるライム病の発生状況に関する報告。1987年に長野で1例目が報告されて以来、日本におけるライム病は主に北海道、本州中部以北で200例以上の確定例の存在が推定される。1995～2000年に北海道で集積したマダニ刺咬症700例のうち確定例が56例(8.0%)であることから、ボレリア汚染地域においてライム病の発症頻度はマダニ刺咬症の10%未満と推定される。また1989～2004年までに113例の確定例を集積した結果、北海道のライム病は皮膚症状が主体で、第II期以後の出現頻度も9例(8.0%)と欧米に比べ低い。また発熱、全身倦怠感などの全身症状の出現頻度も低く、抗菌薬に対する反応も良好で、一般に軽症例が多い。その原因は、ボレリアそのものの病原性の違いや、人種的遺伝的違い、医療状況、マダニの違いなど、複数の要因が関与していると推定される。	21

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献番号
100459	28-Nov-11	110717	バベシア症	CDC Media Relations. Sep 6, 2011	米国におけるバベシア症の輸血感染リスクに関する報告。自覚のないバベシア症感染供血者スクリーニングに対してFDA認可のバベシア検査は利用できない。米国のほとんどのダニ媒介バベシア症は7州において(コネチカット州、マサチューセッツ州、ミネソタ州、ニュージャージー州、ニューヨーク州、ロードアイランド州、ウィスコンシン州)特に暖かい時期に発生している。しかし輸血関連バベシア症は19州において認識され、年間を通して発生している。バベシア症はマラリアと誤診されることがあり、診断が考慮されない限り重症例でも見逃されやすいと指摘される。2011年1月、バベシア症は全国的な届出疾患となり、州保健省はバベシア症例に関して米国疾病管理予防センター(CDC)と情報共有することを奨励した。バベシア症に関する正確な情報を得ることは、血液供給をより安全にするために有益である。	22
100434	24-Oct-11	110642	トリパノソーマ症	第59回日本輸血・細胞治療学会総会; 2011.4.14-16; O-120	在日ブラジル人献血者におけるTrypanosoma cruzi(T. cruzi)抗体検査に関する報告。シャーガス病は中南米で流行し、感染者はT.cruziを長期間体内に保有する無症候性のキャリアとなることが知られている。在日ブラジル人の献血希望者20例についてT.cruzi抗体検査を行ったところ、ELISA法は20例全員陰性であったが、迅速法は19例陰性、1例判定保留であった。追加検査の結果、判定保留の1例は偽陽性であると判断した。年齢は20代9例、30代11例であった。出身地はサンパウロ州17例、パラナ州2例、ミナスジェイラス州1例であった。全員家族にシャーガス病の者はおらず、また過去にT.cruzi抗体検査をした者は1名のみであった。	23
100432	24-Oct-11	110639	異型クロイツフェルト・ヤコブ病	FDA TSEAC 23rd Meeting. Aug 1,2011	FDAの伝達性海綿状脳症諮問委員会(TSEAC)における議題要約書。 FDAは、サウジアラビアにおけるBSEに感染したと考えられる3症例を受け、サウジアラビアでの滞在期間を血液製剤等のドナーの除外条件とすることについてTSEACに助言を求めている。 議題:1)以下の者について血液製剤、組織・細胞由来製剤(HCT/P)のドナーとして不適とすることについて。A) 1980～1996年に米軍としてサウジアラビアに6ヶ月以上滞在した者。B) 1980～1996年にサウジアラビアに累積5年以上滞在した者。 2)血液製剤、HCT/Pの供給及び安全性への上記事項の影響について。 3)更なる安全性基準の必要性について。	24
100494	30-Jan-12	110879	異型クロイツフェルト・ヤコブ病	Haemophilia. 17(2011)931-937	英国の先天性出血性疾患患者におけるvCJD感染リスクに関する報告。英国血友病センター医師機構(UKHCDO)により、供血後vCJDを発症した8人のドナー由来血漿を含む1987-1999年の25バッチの何れかの血液凝固因子製剤の投与を受けた先天性出血性疾患患者におけるvCJD感染リスクが推定された。787例の患者はプロスペクティブに10-20年間調査され、総vCJD感染性は薬剤の総投与量から推測される累積感染性がら推算された。薬剤の投与を受けてから13年以上追跡調査された604例における推定vCJDリスクは595例が1%以上、164例が50%以上、51例が100%という結果であった。これらのリスクが食事によるリスクに上乗せされる。なお、94例はvCJDを発症した患者由来のバッチを供血後6ヵ月以内に投与されていた。2009年1月1日現在、これらの患者でvCJDを発症した患者はいないことは、血漿画分の感染性が過度に見積もられているか、血球製剤の受血者よりも潜伏期間が長いことが原因であると考えられる。	25

医薬品 研究報告 調査報告書

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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

○B型肝炎ウイルスワクチンが無効であった慢性B型肝炎感染
2006年4月、男性パートナーと無防備な性交渉を持つ男性が性病検査のためクリニックを訪れた。HIV、HBV、HCV検査は全て陰性であった。Engerix-B B型肝炎ワクチン(GlaxoSmithKline)接種が実施されたが、2007年8月患者の抗HBs抗体価は10mIU/mL未満であった。ワクチン量が増やされ、2カ月後に抗HBs抗体価は13mIU/mLとなった。2007年11月には抗HBs抗体は2社の検査法で161mIU/mL及び62mIU/mLになったため、ワクチンに対して満足な反応があるとみなされた。2009年12月、患者は疲労感と筋肉痛のため医師を受診した。ALT上昇が見られ、検査によりHBs抗原、抗HBc抗体、HBe抗原が検出された。抗HBs抗体価は10mIU/mL未満であり、加えてHBV DNAが1.7×10^{8}IU/mLを上回るレベルで検出された。系統発生解析によりこのウイルスはHBVジェノタイプFに99%(3214/3215-bp)以上一致した。そのサブジェノタイプF1bは以前アルゼンチン北部で検出され、同性愛男性の間に蔓延していると報告されていた。この患者は2007年と2009年にアルゼンチンを訪れていた。患者は最初抗ウイルス治療は受けず、3カ月毎のフォローアップがされ、2011年の血清学的検査でウイルス量が1.7×10^{8}IU/mL、HBe抗原陽性の慢性HBV感染症であることが確認された。
これは、HBVワクチンを接種され抗HBs抗体価が10mIU/mLを上回る血清学的反応を示した者が、HBVジェノタイプFに感染し、慢性化した初めての報告である。この症例は、ワクチン投与が成功に見えたにも関わらず、HBV感染を防ぐ事ができなかったことを示している。英国や米国を含む多くの国において、ワクチン接種後の抗HBs抗体価が10mIU/mL以上であれば効果があるとされ、ワクチン量の増加は推奨されていない。一方、アイルランドなどではより高い抗体価が必要だとしている。

報告企業の意見	今後の対応
HBVワクチンを受け、抗HBs抗体価が10mIU/mLを上回る血清学的反応を示した者がHBV ジェノタイプFに感染し、慢性化した初めての報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

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Case report

Hepatitis B virus vaccine failure resulting in chronic hepatitis B infection

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1. Case description

A 38 year old asymptomatic male presented to the sexually transmitted infections (STI) clinic in a large teaching hospital in April 2006 for a sexual health screen. He had no significant past medical history although he had engaged in several episodes of unprotected sexual intercourse with male partners in the preceding six months. Serological investigations indicated recent/active *Treponema pallidum* infection for which he was treated. Additional serological investigations for human immunodeficiency virus (HIV), hepatitis B virus (HBV) – comprising hepatitis B surface antigen (HBsAg), antibody to hepatitis B core (anti-HBc) and antibody to surface antigen (anti-HBs) – and hepatitis C virus (HCV) were all negative. A course of Engerix-B® (GlaxoSmithKline) hepatitis B vaccine was commenced and completed at 1 and 6 months following the first dose. In August 2007, the patient's anti-HBs titre was found to be <10 mIU/mL (Table 1). A booster dose was administered and titres measured two months after this were 13 mIU/mL (Abbott Architect). A further booster dose was administered and the anti-HBs titre in November 2007 was 161 mIU/mL and 62 mIU/mL on the Biomerieux VIDAS and Abbott Architect anti-HBs assays respectively. At that time, he was considered to have mounted a satisfactory response to vaccination due to the presence of an anti-HBs titre of >10 mIU/mL in two assays. In December 2009, the patient presented to his primary care physician feeling generally unwell, with non-specific symptoms including fatigue and myalgia. Routine biochemical investigations revealed an elevated alanine aminotransferase (ALT) 211 (5–40) with a normal bilirubin 14 (0–20) and he was referred to the infectious diseases service in a tertiary care hospital for further assessment and management. Serological investigations detected the presence of HBsAg, anti-HBc-specific IgM, and HBeAg, consistent with acute HBV infection (Table 1). Of note, the patient's anti-HBs titre was now <10 mIU/mL. These results were confirmed on a follow-up sample five days later. In addition, HBV DNA (Roche AmpliPrep) was detected at a level of >170 000 000 IU/mL. Serological investigations for HIV and HCV were negative. The patient's entire 3215-bp HBV genome was amplified and sequenced on both strands (Genbank accession number HQ378247).[1] Phylogenetic analysis determined the virus was >99% (3214/3215-bp) similar to a HBV genotype F, sub-genotype F1b previously detected in northern Argentina and a subtype that has previously been reported as circulating in MSM in the country (see Fig. 1).[2,3] The patient had visited Argentina on two occasions in the past between 2007 and 2009. Based on amino acid residues 122K, 160K and 127L of the *S* gene this virus was determined to be the *adw4* serotype.[4] Further analysis of the *S* gene did not reveal the presence of significant vaccine escape mutations such as G145R in the immunodominant 'a' region or any deletions in the *S* gene. The wild-type AGG bases from nucleotides 1762 to 1764 and the wild-type G base at nucleotide position 1896 were observed in the basal core promoter and precore regions of the genome respectively, in keeping with the positive eAg serology.[5] The patient did not receive anti-viral treatment at the time of initial presentation

Abbreviations: Anti HBs antibodies, anti hepatitis B surface antibodies; HBV, hepatitis B virus; STI, sexually transmitted infection; HIV, human immunodeficiency virus; HBsAg, hepatitis B surface antigen; Anti-HBc, hepatitis B core antibody; HCV, hepatitis C virus; ALT, Alanine aminotransferase; HBeAg, hepatitis B e antigen; MSM, men who have sex with men.

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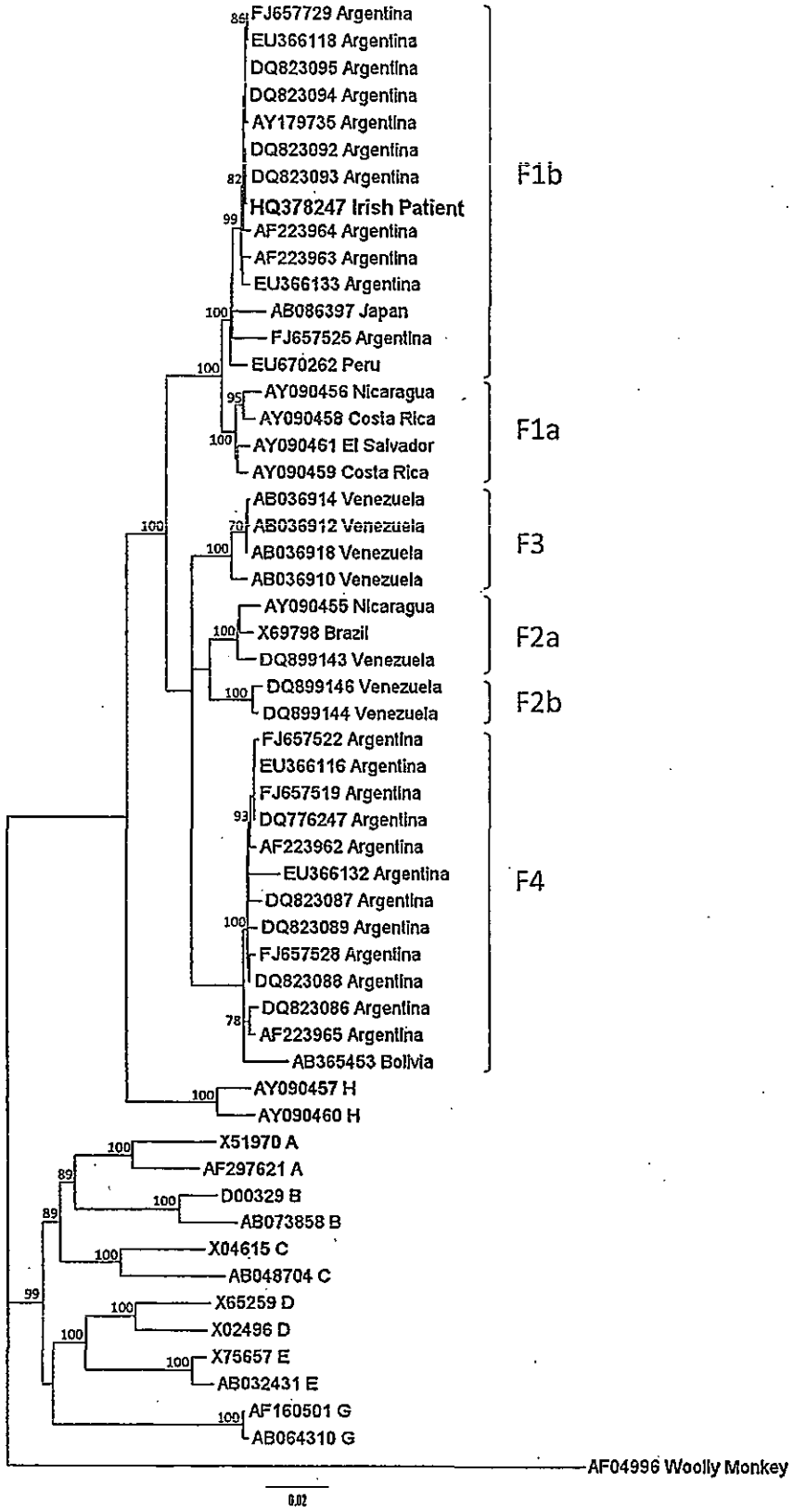

Fig. 1. Neighbour joining phylogenetic tree based on Kimura-two-parameter model on the complete genome of HBV genotypes A–H. Phylogenetic analysis was performed using reference sequences from Genbank, indicated on the tree by their Genbank accession numbers. Woolly Monkey HBV was used as outgroup. Country of origin is included on the nodes of all genotype F reference sequences. The query sequence from this study is represented in boldface. The tree was built in PAUP* (Sinauer Associates, Inc. Publishers). The numbers at the nodes indicate the percentage bootstrap replicates ($n = 1000$) higher than 70%.

Table 1
Development of Hepatitis B serological markers from April 2006 through August 2010.

Date	Vaccine	Anti-HBs	Status
03/04/2006			Negative: HBsAg
21/04/2006		<10 mIU/mL (architect)	
22/01/2007		13.28 mIU/mL (architect)	Negative: HBsAg
29/08/2007		62.79 mIU/mL (architect)161 mIU/mL (Vidas)	Negative: HBsAg, anti-HBc total
08/10/2007			Negative: HBsAg, anti-HBc total
12/11/2007		<10 mIU/mL (architect) 27 mIU/mL (Vidas)	
22/10/2009			Positive weak: HBsAg Negative: HBeAg, HBeAb, anti-HBc total, anti-HBc IgM
17/12/2009		Not detected (architect)	Positive: HBsAg, HBeAg, anti-HBc total, anti-HBc IgM Negative: HBeAb
22/12/2009			Positive: HBsAg, HBeAg, anti-HBc total, anti-HBc IgM, DNA (>170 000 000 IU/mL) Negative: HBeAb
09/02/2010		Not detected (architect)	Positive: HBsAg, HBeAg, anti-HBc total, anti-HBc IgM, DNA (98 589 232 IU/mL) Negative: HBeAb
03/03/2010			Positive: HBsAg, HBeAg, anti-HBc total, anti-HBc IgM Negative: HBeAb
26/08/2010			Positive: HBsAg, HBeAg DNA (>170 000 000 IU/mL) Negative: HBeAb, anti-HBc IgM

and was followed up at 3 monthly intervals. Subsequent serological investigation in June 2011 confirmed HBeAg-positive chronic HBV infection with a viral load of >170 000 000 IU/mL.

2. Why this case is important

This report describes, what is to the authors' knowledge, the first case of HBeAg-positive chronic HBV infection (genotype F) occurring in an individual who received several doses of HBV vaccine and demonstrated an anti-HBs serological response of >10 mIU/mL, the accepted threshold for protection.[6,7] This finding suggests this patient was not protected from chronic HBV despite seemingly successful vaccination. There is ongoing debate regarding the post vaccine anti-HBs response necessary to protect against HBV infection. Many countries (including the United Kingdom and the United States) use a value of >10 mIU/mL as a threshold to determine immunity and do not routinely recommend booster doses of vaccine, whereas others (including Ireland) require higher levels (>100 mIU/mL) of anti-HBs to indicate protection.[8] While the duration of long term immunity post vaccination is unclear, recent meta-analyses suggests it lasts for up to twenty years in immune competent patients.[9–13]

3. Other similar and contrasting cases in the literature

Primary HBV vaccine failure is not uncommon among adults[14] with studies estimating that up to 10% of immune competent adults do not respond to HBV vaccine.[15,16] However, very few cases of vaccine failure have been documented in immune competent individuals who have an adequate anti-HBs response.[17,18] A previous report described acute HBV genotype A infection 14 years post successful vaccination,[17] although arguably this report does not constitute vaccine failure. On the contrary, the report of Boot and colleagues actually serves to demonstrate the long-term effectiveness of vaccination in protecting against chronic HBV infection.[17] Similarly, acute HBV genotype F has previously been reported in a successfully vaccinated (Twinrix®) immune competent German patient.[18] Importantly, no HBsAg escape mutations were detected, and the infection resolved.[18] It is known that in highly vaccinated populations, individuals vaccinated against HBV may become infected. However, they do not typically progress to chronic HBV infection even in the setting of waning anti-HBs titres, as immunologic memory confers sufficient immunity.[19] Nevertheless, in the case reported herein, two years following seemingly successful vaccination, a young homosexual male presented with acute genotype F HBV infection that progressed into chronicity. It is unclear why this patient should have developed chronic HBV infection, although acknowledged the final anti-HBs levels remained relatively low despite five doses of vaccine.

4. Discussion

Current vaccination for HBV utilises a recombinant antigen targeting the immunodominant 'a' determinant region of the *S* gene of HBV. The Engerix-B® vaccine used in this case employs the recombinant small envelope S protein of HBV genotypes A and D, the most common circulating genotypes in Ireland, Europe and North America. However, sequence differences exist in this region between HBV genotype F viruses and genotypes A and D.[18] Indeed, genotype F is one of the most genetically diverse HBV genotypes. It is found predominantly in Central and South America[20] and rarely in Ireland[21] (unpublished data). The detection of a genotype F strain is in keeping with the sexual history of the present case, who reported multiple unprotected sexual encounters in Argentina. HBV Genotype F also has been associated with acute (typically HBeAg-positive) symptomatic infections in Argentina,[22] and therefore higher viral loads. Thus, it could be suggested that repeated MSM sexual exposure to this genotype and the high viral load encountered by the patient may have overwhelmed any protection conferred by the sub-optimal anti-HBs level. Nevertheless, Pezzano and co-workers reported that genotype F was more commonly associated with acute than chronic infection and therefore the current case is surprising.[22] One potential alternative explanation for the case described is that the initial anti-HBs results were incorrect. This is unlikely. Firstly, testing was performed in an accredited laboratory using internal quality controls to monitor assay performance: in addition, external quality assurance distributions tested over the same time period achieved 100% concordance with expected results. Secondly, despite minor inter-assay variation in the absolute anti-HBs titres obtained, the Architect and Vidas assays each confirmed the results of the other. Thirdly and finally, 3 sequential samples were tested for anti-HBs over a 4 month period, all generating results compatible with the patient's vaccination history. Following these initial results, a decline in anti-HBs over the subsequent 24 months was noted. This decline is not exceptional as levels of vaccine-induced antibody are known to wane over time.[23] Indeed, post-vaccine screening is typically performed 2–4 months following completion of the

course of vaccine to ensure accurate anti-HBs measurement before waning ensues. To the authors' knowledge, this is the first report of a patient developing chronic HBV infection with a genotype F virus following an apparently adequate anti-HBs response. The patient was otherwise healthy and immune competent; no known escape mutations were identified in the viral genome; and it is established that the HBV vaccine protects against chronic infection in the immunocompetent individual. However, this case illustrates a scenario in which the level of protection conferred from a sub-optimal vaccine-induced immune response may not protect against significant challenge with a high viral load heterotypic HBV genotype infection. Therefore it may be necessary to review the protective level in those groups, such as MSM, who may be at increased risk of exposure to HBV infected individuals with high viral loads.

Funding

None.

Competing interest

None declared.

Ethical approval

Not required.

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-15- 研究報告の概要

業界のためのガイダンス
— 全血および血液成分(原料血漿を含む)のドナーから得られた個々のサンプルおよびそれらのプールについての、B 型肝炎ウイルス伝播危険性を低減させるための核酸検査の使用について —

ガイダンス案

FDA(米国食品医薬品庁)は、輸注またはそれを原料として製造するために全血または血液成分(それには、リカバード血漿、原料血漿、および原料白血球を含むが)を採取する血液採取・取扱施設に対する、FDA 認可済の核酸増幅検査(NAT)を B 型肝炎ウイルス(HBV)デオキシリボ核酸(DNA)の有無について血液ドナーをスクリーニングすることに関しての勧告事項をここに提示する。また、FDA は製品試験とその処置、ドナー管理、ドナーの再適格化、および製品の表示についての勧告事項も、血液採取・取扱施設に提示する。

(中　略)

IV. 勧告事項

A. HBV NAT を用いたドナースクリーニング

§610.40(b)に従って、血液採取・取扱施設は FDA がスクリーニング検査用として認可したスクリーニング法を、製造者の使用説明書に従って用いなければならない。血液採取・取扱施設は、HBV を含む伝染性疾患の伝播の危険性を十分かつ適切に低減させるために必要に応じてそのような検査を 1 種類以上行わなければならない。

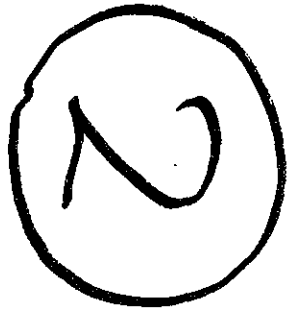
1. 輸注用の全血および血液成分、ならびに製造用の原料白血球についての§610.40(b)に規定されている要求事項に合致させるために、FDA は血液採取・取扱施設が、HBsAg と抗 HBc の検出に加えて、FDA が認可した NAT による HBV DNA についてのドナースクリーニング試験を用いることを勧告する。HBsAg および抗 HBc の検出用として FDA が認可した検査法で陰性もしくは無反応であった場合には、FDA は血液採取・取扱施設が、個々のドネーション中の HBV DNA 検出用として検出下限が＜100 IU/mL HBV DNA であるような FDA が認可済の HBV NAT を用いてドネーションをさらに試験することを勧告する。血液採取・取扱施設の用いる FDA 認可済の HBV NAT によるスクリーニングは、ミニプールを用いたドネーションサンプル試験フォーマットで行うことができ、または個々のドネーションを検査するフォーマットで行うこともでき、他のもの、例えば HIV や HCV の検査とともに多重 NAT とすることができ、または HBV のみを検査する NAT とすることができる。HBsAg、抗 HBc、および HBV DNA の NAT による検査は同時に行うことができる。

2. 血漿分画製剤への製造用の原料血漿の検査について§610.40(b)の要求事項に合致させるために、FDA は血液採取・取扱施設が HBsAg 検出用に FDA が認可済のドナースクリーニング検査を用いることを勧告する。HBsAg 検出用の FDA 認可済検査

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意

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



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法で検査して陰性または無反応の場合には、FDA は血液採取・取扱施設が当該ドネーションをさらに、個々のドネーション中の HBV DNA 検出用として検出下限が＜500 IU/mL HBV DNA であるような FDA が認可済の HBV NAT を用いて検査することを勧告する。血液採取・取扱施設の用いる FDA 認可済の HBV NAT によるスクリーニングは、ミニプールを用いたドネーションサンプル試験フォーマットで行うことができ、または個々のドネーションを検査するフォーマットで行うこともでき、他のもの、例えば HIV 検査や HCV 検査とともに多重 NAT とすることができ、または HBV のみを検査する NAT とすることができる。HBsAg および HBV DNA の NAT による検査は同時に行うことができる。(FDA は現在のところ、原料血漿のドナーに抗 HBc 検査を行うことは勧告していない(参照文献 2))。

（後　略）

報告企業の意見	今後の対応
米国で全血および血液成分について HBV NAT を導入するとの FDA のガイダンス（案）である。弊社血漿分画製剤の原料血漿については、以前より HBV のミニプール NAT を導入している。 HBV は直径 42nm の球形をした DNA ウイルスで、ウイルス粒子は二重構造をしており、ウイルス DNA をヌクレオカプシドが包む直径約 27nm のコア粒子と、これを被うエンベロープから成り立っている。万一、原料血漿に HBV が混入したとしても、BVD 及び BHV をモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

ハプトグロビン

Guidance for Industry

Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components, including Source Plasma, to Reduce the Risk of Transmission of Hepatitis B Virus

DRAFT GUIDANCE

This guidance is for comment purposes only.

Submit one set of either electronic or written comments on this draft guidance by the date provided in the *Federal Register* notice announcing the availability of the draft guidance. Submit electronic comments to http://www.regulations.gov. Submit written comments to the Division of Dockets Management (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. You should identify all comments with the docket number listed in the notice of availability that publishes in the *Federal Register*.

Additional copies of this guidance are available from the Office of Communication, Outreach and Development (OCOD) (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or e-mail ocod@fda.hhs.gov, or from the Internet at http://www.regulations.gov, http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/default.htm.

For questions on the content of this guidance, contact OCOD at the phone numbers or e-mail address listed above.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2011

Contains Nonbinding Recommendations

Table of Contents

Contains Nonbinding Recommendations

Guidance for Industry

Use of Nucleic Acid Tests on Pooled and Individual Samples from Donors of Whole Blood and Blood Components, including Source Plasma, to Reduce the Risk of Transmission of Hepatitis B Virus

This draft guidance, when finalized, will represent the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

We, FDA, are providing you, blood establishments that collect Whole Blood and blood components for transfusion or for further manufacture, including recovered plasma, Source Plasma and Source Leukocytes; with recommendations concerning the use of FDA-licensed nucleic acid tests (NAT) to screen blood donors for hepatitis B virus (HBV) deoxyribonucleic acid (DNA). We are also providing you with recommendations for product testing and disposition, donor management, methods for donor requalification, and product labeling.

In addition, we are notifying you in this guidance that we consider the use of an FDA-licensed HBV NAT to be necessary to reduce adequately and appropriately the risk of transmission of HBV. FDA-licensed HBV NAT can detect evidence of infection at an earlier stage than is possible using previously approved hepatitis B surface antigen (HBsAg) and antibody to hepatitis B core antigen (anti-HBc) tests. Therefore, we recommend that you use FDA-licensed HBV NAT, in accordance with the requirements under Title 21 Code of Federal Regulations, 610.40(a) and (b), (21 CFR 610.40(a) and (b)).

This guidance supplements previous memoranda and guidance from FDA to blood establishments concerning the testing of donations for HBsAg and anti-HBc, and the management of donors and units mentioned in those documents (Refs. 1 through 5). Note that testing Whole Blood and blood components for transfusion and Source Leukocytes for further manufacture for HBsAg and anti-HBc, and Source Plasma for HBsAg should continue when a blood establishment implements HBV NAT. [1] FDA may consider advancements in technology

[1] FDA does not currently recommend that Source Plasma donors be tested for anti-HBc. If anti-HBc reactive units were excluded from pools used for the manufacture of plasma derivatives, titers of neutralizing antibody to hepatitis B surface antigen (anti-HBs) in those pools would be expected to diminish, as both these antibodies usually occur together. The presence of neutralizing anti-HBs is believed to contribute to the safety of certain plasma products. (Ref. 2). Plasma units that are untested, non-reactive (NR), or repeat reactive (RR) for anti-HBc are currently acceptable for the manufacture of plasma derivatives (Ref. 2). Consistent with § 610.40(h)(2)(v), recovered plasma

for testing blood donations, as well as data obtained following the implementation of HBV NAT, to make future recommendations on adequate and appropriate testing for HBV.

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe the FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. DEFINITIONS

Discriminatory NAT: A NAT that uses specific primers for HIV-1 or HBV or HCV to identify the RNA or DNA in the reactive multiplex NAT sample as HIV-1 RNA or HBV DNA or HCV RNA. Performing a Discriminatory NAT on a reactive sample is a required step for those establishments using an approved multiplex test. The labeling for licensed multiplex NATs specifies that Discriminatory NAT is to be performed. Under § 610.40(b) (21 CFR 610.40(b)), you must use FDA-approved screening tests in accordance with the manufacturer's instructions.

Donor Reentry: A procedure that qualifies a deferred donor as eligible to donate again. Donor reentry procedures may be used following a false positive test result and typically require the passage of time to allow for possible seroconversion prior to the performance of additional serologic testing and NAT.

HBV NAT assay with a limited supplemental test indication: Some HBV NAT assays have received a limited supplemental indication for repeatedly reactive HBsAg test results. If a donation tests HBV NAT-positive for HBV DNA using an HBV NAT with such a limited supplemental test indication, and if that donation also tests HBsAg repeatedly reactive in a screening test, the HBsAg test result can be recorded as HBsAg positive. In this case, an HBsAg neutralization test need not be performed. However, if a donation tests HBV NAT-negative for HBV DNA using an HBV NAT with such a limited supplemental test indication, and if that donation tests HBsAg repeatedly reactive in a screening test, an HBsAg neutralization test should be performed. In this case, the result of the neutralization test serves as the test of record. (Ref. 1)

Minipool: A pool of donor samples on which NAT (minipool NAT or MP-NAT) is performed as a screening test. A minipool is formed by pooling of samples from subpools or by directly pooling samples from individual donors.

Multiplex NAT: A NAT that simultaneously detects HIV-1 RNA, HBV DNA, and HCV RNA.

Single Virus NAT: A NAT that separately detects either HIV-1 RNA or HBV DNA or HCV RNA.

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from donations of Whole Blood that test anti-HBc reactive may be used for further manufacture into plasma derivatives.

III. BACKGROUND

Under § 610.40(a), establishments that collect blood or blood components must test each donation of human blood or blood component intended for use in preparing a product, including donations intended as a component of, or used to prepare a medical device, for evidence of infection due to certain communicable disease agents, including HBV. In addition, under § 610.40(b), you must perform one or more such tests as necessary to reduce adequately and appropriately the risk of transmission of communicable disease.

Currently, all Whole Blood and blood components intended for transfusion and all Source Leukocytes intended for further manufacture are routinely tested for HBsAg and anti-HBc in order to reduce the risk of transmission of HBV (Refs. 1, 2, 3 and 5). In addition, all Source Plasma collections intended for further manufacture into plasma derivatives are routinely tested for HBsAg in order to reduce the risk of transmission of HBV in manufacturing pools of plasma derivatives.[2]

In the preamble to the final rule entitled "Requirements for Testing Human Blood Donors for Evidence of Infection Due to Communicable Disease Agents," published in the *Federal Register* of June 11, 2001 (66 FR 31146), we discussed the approved donor screening tests that we considered, as of that date, to be necessary to reduce adequately and appropriately the risk of transmission of HBV. We also stated that as technology advances, we intend to issue guidance describing those tests that we consider to reduce adequately and appropriately the risk of transmission of communicable disease agents. Accordingly, in this draft guidance document, we are notifying you that we consider FDA-licensed HBV NAT to be necessary to reduce adequately and appropriately the risk of transmission of HBV.

We note that the tests referenced in this document have been licensed by FDA for the screening of blood donors for HBV DNA and have the ability to detect the evidence of infection at an earlier stage than is possible using previously approved HBsAg and antibody to hepatitis B core antigen (anti-HBc) tests. Because FDA-licensed HBV NAT are now widely available, we recommend that establishments use these tests, in accordance with § 610.40.

A. Rationale for Donor Screening Using HBV NAT

Hepatitis B virus is a major human pathogen that may cause acute and chronic hepatitis, cirrhosis and hepatocellular carcinoma (Ref. 6). Most primary infections in adults are self-limited, the virus is cleared from blood and liver, and individuals develop a lasting immunity. Fewer than 5% of infected adults develop persistent asymptomatic infections (i.e., a carrier state). However, infants and young children have a much higher likelihood of developing a chronic hepatitis B infection than do older children and adults. According to data obtained in 2004 from the Centers for Disease Control and Prevention, about 1% of adults without other preexisting conditions are estimated to get chronic hepatitis B if infected, but 2% to 10% of children more than 5 years of age get chronic

[2] See Footnote 1.

hepatitis B, and 30% to 90% of children less than 5 years of age develop chronic hepatitis B if infected (Ref. 7). In addition, many patients receiving blood are immunocompromised because of their underlying disease and/or because of medications that suppress the immune system making them more susceptible to severe HBV infection than otherwise healthy individuals. About 20% of chronically infected individuals can develop cirrhosis. Chronically infected subjects have 100 times higher risk of developing hepatocellular carcinoma than non-carriers (Ref. 6).

Currently, HBV is transmitted by blood transfusions more frequently than hepatitis C virus (HCV) or human immunodeficiency virus (HIV). The residual risk of post-transfusion HBV infection is estimated to be about 1:357,000 to 1:280,000 per transfusion. In comparison, those risks for HIV and HCV are estimated to be 1:1,467,000 and 1:1,149,000, respectively (Ref. 8). Depending on the sensitivity of the test, implementation of HBV NAT has the potential to reduce risk to levels similar to those for HIV and HCV. HBV can be transmitted by blood from asymptomatic donors with acute HBV infections who have not yet developed HBsAg or anti-HBc (i.e., donors in the seronegative window period), when HBV DNA can be detected in the donor's blood (Refs. 9 and 10). Depending on the relative sensitivities of HBsAg and HBV NAT assays used, HBV DNA can be detected 2 to 5 weeks after infection, and up to 40 days (mean 6 to 15 days) before HBsAg (Ref. 7). HBV DNA levels rise slowly and are present at relatively low levels during the seronegative window period of early infection. HBV DNA can also be detected along with HBsAg and anti-HBc in chronic hepatitis B infections, and sometimes in recovered infections that are negative for HBsAg and positive for antibodies to hepatitis B surface antigen (anti-HBs) and anti-HBc (Refs. 6 and 11). Rarely, HBV DNA can be detected in the absence of HBsAg, anti-HBc and anti-HBs (Ref. 12).

Blood for transfusion in the United States (U.S.) is also tested for anti-HBc. Anti-HBc develops a few days after the appearance of HBsAg and usually remains detectable for life, irrespective of whether the individual recovers from acute hepatitis B or whether chronic HBV infection develops. Because of the availability and use of tests to detect anti-HBc, HBV NAT's potential utility in further reducing risk of hepatitis B transmission by blood transfusion is mainly restricted to the early HBsAg-negative phase of infection (i.e., a potential reduction of the infectious window period of up to 40 days depending on sensitivity of the HBsAg test).

There are currently three FDA-licensed HBV NAT assays for screening Whole Blood and blood components available in the U.S. Following licensure of the first HBV NAT assay in April 2005 (the Roche COBAS AmpliScreen HBV NAT that uses pools of up to 24 donation samples), FDA did not recommend use of HBV NAT. At that time, FDA's position on the use of HBV NAT was based, in part, upon discussions by the Blood Products Advisory Committee (BPAC or Committee) at the meeting on July 23, 2004 (Ref. 13), and on a recommendation from the Department of Health and Human Services Secretary's Advisory Committee on Blood Safety and Availability (ACBSA) on August 27, 2004 (Ref. 14). In making its recommendations, the ACBSA considered a number of broad public health issues including cost-effectiveness, feasibility, and overall public

health benefit, in addition to scientific data on detection of HBV in donors. One of FDA's reasons for not recommending HBV NAT at that time was the sensitivity of HBV NAT in the available format, when compared to the available serologic testing, did not provide sufficient additional safety to the blood supply to warrant recommending its use. FDA's reasoning was based on information that most blood establishments would have to test pools of 24 samples (thus diluting the individual samples by 1:24), because it was not feasible for most blood establishments to test single samples from donations or even small pools of samples.

Since licensure of the first HBV NAT in 2005, the following changes have occurred:

1. FDA has licensed two additional HBV NAT assays with indications for blood donor screening: Procleix® ULTRIO® Assay (Gen-Probe, Inc., San Diego, California), which uses up to 16 donation samples in a pool and COBAS TaqScreen MPX Test (Roche Molecular Systems, Inc., Pleasanton, California), which uses up to 6 donation samples in a pool. These multiplex assay systems can simultaneously detect HIV, HCV and HBV in a single donation, thus improving the feasibility of routine NAT testing for HBV. FDA has also licensed the UltraQualTM HBV PCR Assay (National Genetics Institute, Los Angeles, California), which provides results of HBV NAT of Source Plasma samples, or of plasma samples from Source Plasma donors at the time of donation. The assay is an "in-house" test; no kit is sold. The assay uses up to 512 donation samples in a pool.)

2. With the recent advance in technology and increased automation enabling the performance of NAT with smaller pools of samples and individual samples, more sensitive HBV NAT testing of blood donations is now possible, resulting in an increase in the number of window period HBV DNA positive/HBsAg negative units that could be detected.

3. There is now more information available on the role of vaccination of donors and recipients against HBV infection that indicates that protection for the long term is not absolute (i.e., breakthrough infections can occur in previously vaccinated individuals who are exposed to the virus) (Refs. 10 and 15). Breakthrough infections are characterized by HBV NAT positivity, the presence of HBV-neutralizing anti-HBs (developed as a result of hepatitis B vaccination), low viral load and lack of symptoms. HBsAg and anti-HBc may not subsequently develop or their appearance may be delayed. The infectivity of units obtained from hepatitis B-vaccinated donors with breakthrough HBV infections is unknown at the present time.

As mentioned above, in breakthrough HBV infections, HBsAg and anti-HBc development may be delayed or might not occur. Development is more likely to be detected by HBV NAT, particularly in the early stages of infection. As younger cohorts

in the population, who have received hepatitis B vaccine in a greater proportion than older cohorts (Refs. 16, 17, and 18), become eligible to donate blood, the proportion of vaccinated donors compared to non-vaccinated donors is expected to increase. Therefore, the proportion of donors with HBV breakthrough infections, compared to those with non-breakthrough, wild-type, HBV infections, would also be expected to increase. These donors' asymptomatic breakthrough infections are more likely to be detected by HBV NAT than to be detected by HBsAg or anti-HBc assays because HBsAg and anti-HBc development might be delayed or might not occur, even though HBV DNA is present and detectable by HBV NAT in the initial stage of the infection. In addition, HBV mutants appear to be more likely to be detected by HBV NAT than by HBsAg assays (Ref. 10).

Much of the available literature seem to indicate that HBV NAT positive/anti-HBs positive/HBsAg negative blood, irrespective of anti-HBc test results, does not transmit HBV (Refs. 11, 12, 19, 20, 21, and 22). However, there are at least two reports of such possible transmissions (Refs. 23 and 24), and one report that appears to have confirmed transmission of HBV by HBV NAT positive/anti-HBs positive/HBsAg negative blood (Ref. 25). Therefore, there can be no assumption of non-infectivity of units from donors with breakthrough infections containing HBV DNA and vaccine-induced, HBV-neutralizing anti-HBs when transfused into recipients. Nor can we assume a lack of morbidity and mortality in recipients, especially when many recipients are immunocompromised, as previously mentioned.

At the April 1, 2009 BPAC meeting (Ref. 26), the Committee agreed with FDA's position that there is no assumption of non-infectivity to recipients of units from donors with breakthrough infections. Therefore, in this guidance, we are recommending that all units of blood used for transfusion should be tested by an FDA-licensed HBV NAT. The Committee also supported FDA setting a sensitivity standard of 200 IU/mL HBV DNA for detection of HBV DNA in an individual donation when HBV NAT assays are used to test blood and blood components intended for transfusion. However, because of technological advances that have occurred since the time of the BPAC meeting in 2009, we are recommending a sensitivity standard of 100 IU/mL for HBV DNA detection in an individual donation (see section IV.A). Due to advances in technology and automation, FDA considers a sensitivity standard of 100 IU/mL to be attainable and practical for blood establishments that collect donations of Whole Blood and blood components intended for transfusion.

With regard to testing Source Plasma units for further manufacture into injectable plasma derivatives for HBV DNA, we believe that such testing adds another layer of safety for plasma derivatives by limiting the viral load in plasma pools for fractionation, in addition to viral inactivation and/or removal steps during their manufacture and the presence of neutralizing anti-HBs in manufacturing pools. During the BPAC meeting held on April 28, 2011 (Ref. 27), the Committee agreed with FDA that the available scientific data supports the concept that testing Source Plasma donations by HBV NAT increases the safety margin of plasma derivatives. Therefore, FDA is recommending that all units of Source Plasma intended for manufacture into injectable plasma derivatives be tested by

an FDA-licensed HBV NAT. In consideration of viral inactivation and removal in plasma fractionation, FDA is recommending a sensitivity standard of 500 IU/mL for detection of HBV DNA in an individual collection, rather than 100 IU/mL (see section IV.A). This sensitivity standard was endorsed by BPAC at the April 28, 2011 meeting (Ref. 27).

Similar to plasma derivatives, the HBV safety of products made from Source Leukocytes depends in large measure on viral removal and inactivation during manufacturing. However, since Source Leukocytes are obtained from Whole Blood donors, for consistency, we are also recommending a sensitivity standard of 100 IU/mL for HBV DNA detection in the individual donation of Source Leukocytes.

B. Donor Requalification

Under § 610.41(b), "[a] deferred donor subsequently may be found to be suitable as a donor of blood or blood components by a requalification method or process found acceptable for such purposes by FDA."[3]

At the July 21, 2005 BPAC meeting (Ref. 28), the Committee agreed with FDA's proposed requalification criteria for donors of Whole Blood and blood components for transfusion and Source Plasma for further manufacture, who tested reactive by HBV NAT, when a follow-up sample is tested using HBV NAT and serologic tests. Data presented at the meeting demonstrated that a 6-month follow-up period encompasses the pre-seroconversion window period with sufficient confidence that negative test results for HBsAg, anti-HBc and HBV DNA by NAT, after a 6-month period, rule out HBV infection. For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT labeled as having a sensitivity of $\leq$2 IU/mL at 95% detection rate [1 IU = ~5 copies of HBV DNA/mL].[4] Donors with negative results for HBV DNA at this level of sensitivity are highly unlikely to be infected with HBV (Ref. 29). Depending upon the assay and the platform used, this sensitivity may only be achieved when testing individual donor samples. Recommended criteria for donor requalification are presented in section IV.C.

IV. RECOMMENDATIONS

A. Donor Screening Using HBV NAT

Under § 610.40(b), you must use screening tests that FDA has approved for such use, in accordance with the manufacturers' instructions. You must perform one or more such

[3] A deferred donor may serve as an autologous donor in accordance with § 610.40 and § 610.41. Note that a deferred donor who donates for autologous use is not deemed to be reentered and remains deferred, until the criteria for reentry are met.

[4] COBAS AmpliScreen HBV Test (Roche Molecular Systems, Inc., Pleasanton, California): Triplicate testing using the multiprep specimen processing procedure. See package insert.
Procleix® ULTRIO® Assay (Gen-Probe, Inc., San Diego, California): Testing 6 replicates. See package inserts.

tests as necessary to reduce adequately and appropriately the risk of transmission of communicable disease, including HBV.

1. In order to meet the requirement under § 610.40(b) for Whole Blood and blood components intended for transfusion and Source Leukocytes intended for further manufacture, we recommend that you use an FDA-licensed donor screening test for HBV DNA by NAT in addition to the detection of HBsAg and anti-HBc. If the FDA-licensed tests for detection of both HBsAg and anti-HBc are negative or non-reactive, we recommend that you test the donation further using an FDA-licensed HBV NAT that has a lower limit of detection of <100 IU/mL HBV DNA for HBV DNA detection in an individual donation. The FDA-licensed screening HBV NAT that you use may be in a minipool donation-sample testing format or an individual donation testing format, and may include multiplex NAT with testing of other agents, such as HIV and HCV or may be single virus NAT for HBV only. Testing for HBsAg, anti-HBc and HBV DNA by NAT may be performed concurrently.

2. In order to meet the requirement under § 610.40(b) for testing Source Plasma intended for further manufacture into plasma derivatives, we recommend that you use an FDA-licensed donor screening test for the detection of HBsAg. If the FDA-licensed test for detection of HBsAg is negative or non-reactive, we recommend that you test the donation further using an FDA-licensed HBV NAT that has a lower limit of detection of <500 IU/mL HBV DNA for HBV DNA detection in an individual donation. The FDA-licensed screening HBV NAT that you use may be in a minipool donation-sample testing format or an individual donation testing format, and may include multiplex NAT with testing of other agents, such as HIV and HCV, or may be single virus NAT for HBV only. Testing for HBsAg and HBV DNA by NAT may be performed concurrently. (FDA does not currently recommend that Source Plasma donors be tested for anti-HBc (Ref. 2)).

   As a general matter, under § 610.40(h)(1), if any of the FDA-licensed tests for the detection of either HBsAg or anti-HBc is reactive, the donation must be not be shipped or used.[5] In this instance, we believe that you have met the standard for adequate and appropriate screening for HBV and you do not need to test the unit further using an FDA-licensed HBV NAT. However, you may choose to test such a reactive donation by using an FDA-licensed HBV NAT to provide useful information to the donor, or if you wish to reenter the donor as described below in this Guidance.

   We note that in regard to HBsAg reactivity, as required by § 610.40(e), you must proceed to supplemental testing for HBsAg to determine

[5] Blood components that are reactive for HBsAg and/or anti-HBc may be shipped or used if they meet the conditions for an exception described in § 610.40(h)(2).

whether or not a reactive HBsAg test result can be confirmed positive, and is not a false positive (i.e., test result recorded HBsAg negative), using either an additional, more specific test, such as an HBsAg neutralization test or an HBV NAT assay with a limited supplemental test indication. Some HBV NAT assays have received this limited supplemental indication for repeatedly reactive HBsAg test results. If a donation tests HBV NAT-positive for HBV DNA using an HBV NAT with a limited supplemental test indication, and if that donation also tests HBsAg repeatedly reactive in a screening test, the HBsAg test result can be recorded as HBsAg positive. In this case, an HBsAg neutralization test need not be performed. However, if a donation tests HBV NAT-negative for HBV DNA using an HBV NAT with a limited supplemental test indication, and if that donation tests HBsAg repeatedly reactive in a screening test, an HBsAg neutralization test should be performed. In this case, the result of the neutralization test serves as the test of record (Ref. 1). We further note that there is no licensed supplemental, more specific, test for anti-HBc at the present time. Donors with anti-HBc reactive results may be requalified as described in Ref 3.

B. Management of Donors and Units Based on Hepatitis B Test Results

1. Donor and Unit Management When the HBV DNA NAT Result is Negative

   a. If a unit tests negative by individual donation NAT (ID-NAT) for HBV DNA or is part of a minipool that tests negative, then the donor and the unit should be managed consistent with FDA guidances and recommendations, as appropriate (Refs. 1 through 5), provided that the donor satisfies all applicable donor eligibility criteria and the unit is otherwise suitable for release.

   b. Units of Whole Blood and blood components may be used for transfusion and Source Leukocytes may be used for further manufacture that test negative for HBV using FDA-licensed HBV NAT, HBsAg, and anti-HBc assays, provided that the donor satisfies the donor eligibility criteria in § 640.3 (21 CFR 640.3), and that all other donor screening tests for communicable disease agents required in § 610.40(a) and (i) for Whole Blood and blood components, including Source Leukocytes, are negative, and the units are otherwise suitable for release.

   c. Units of Source Plasma and recovered plasma that test negative for HBV using FDA-licensed HBV NAT and HBsAg assays may be used for further manufacture, provided that the donor satisfies the donor eligibility criteria in § 640.63 (for Source Plasma) and § 640.3 (for recovered plasma), and that the requirements in § 610.40 are met and

all other screening tests for communicable disease agents required in § 610.40(a) and (i) are negative and the units are otherwise suitable for release (see footnote 1).

2. Donor and Unit Management when the HBV DNA NAT Result is Positive

   a. In accordance with § 610.40(h), except for autologous donations under § 610.40(h)(2)(i) or where you have obtained FDA's written approval for the shipment or use in accordance with § 610.40(h)(2)(ii)(A), you must not ship or use a unit of Whole Blood or blood components for transfusion, or a unit of Source Leukocytes for further manufacture that tests positive by HBV ID-NAT (either from direct screening by ID-NAT or from deconstruction of a NAT-positive minipool) (Table 1, Categories 1 through 6).

   b. In accordance with § 610.41, you must defer a donor who tests reactive for HBV, and in accordance with 21 CFR Part 630 (Part 630) you must notify the blood donor. You should permanently defer a donor of Whole Blood or blood components, or Source Leukocytes, whose NAT and serologic test results are as follows. (The donor is not eligible for reentry):

      i. HBV NAT-positive, HBsAg RR and confirmed positive, either by neutralization or when using a NAT with a limited supplemental claim, regardless of anti-HBc results (Table 1, Categories 1 and 2); or

      ii. HBV NAT-positive when using a NAT that does not have a limited supplemental claim and HBsAg RR is not confirmed by neutralization, and anti-HBc is RR (Table 1, Category 3).

   c. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with Part 630 you must notify the blood donor. You should indefinitely defer a donor of Whole Blood or blood components, including Source Leukocytes, whose NAT and serologic test results are as follows (The donor may be eligible for reentry, as described in section IV.C.):

      i. HBV NAT-positive, HBsAg non-reactive (NR), anti-HBc RR (Table 1, Category 4); or

      ii. HBV NAT-positive, and both HBsAg and anti-HBc are non-reactive (Table 1, Category 5); or

iii. HBV NAT-positive using a NAT that does not have a limited supplemental claim and HBsAg RR is not confirmed by neutralization, and is anti-HBc NR (Table 1, Category 6).

Table 1. Donor and Unit Management (Whole Blood and Blood Components for transfusion, and Source Leukocytes for Further Manufacture) when the HBV DNA NAT Result is Positive

Category	HBV NAT Result†	HBsAg Result	Anti-HBc Result	Donor and Unit
1	Positive	Repeat Reactive / Confirmed Positive*	Non-Reactive	Discard unit; Permanently defer donor Donor not eligible for reentry
2	Positive	Repeat Reactive / Confirmed Positive*	Repeat Reactive	
3	Positive	Repeat Reactive / Not Confirmed	Repeat Reactive	
4	Positive	Non-Reactive	Repeat Reactive	Discard unit; Indefinitely defer donor; Donor may be eligible for reentry
5	Positive	Non-Reactive	Non-Reactive	
6	Positive	Repeat Reactive / Not Confirmed	Non-Reactive	

† Using a screening test, as described in section IV.A.1.
*Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section III and section IV.A.3.

d. In accordance with § 610.40(h), except where you have obtained FDA's written approval for the shipment or use in accordance with § 610.40(h)(2)(ii)(A), you must discard and not use for further manufacture a unit of Source Plasma that tests positive by HBV ID-NAT (Table 2, Categories 1 through 3).

e. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with Part 630 you must notify the blood donor. You should permanently defer a donor of Source Plasma whose donation tests HBV NAT-positive and is HBsAg RR, confirmed positive either by neutralization, or when using a NAT with a limited supplemental claim. The donor is not eligible for reentry (Table 2, Category 1).

f. In accordance with § 610.41, you must defer a donor who tests reactive for tests for HBV, and in accordance with 21 CFR 630 you must notify the blood donor. You should indefinitely defer a donor of Source Plasma whose donation tests HBV NAT-positive when using a NAT that does not have a limited supplemental claim, and is either HBsAg NR or is HBsAg is RR not confirmed by neutralization (Table 2, Categories 2 and 3). The donor may be eligible for reentry, as described in section IV.C.

Table 2. Donor and Unit Management (Source Plasma for Further Manufacture) when the HBV DNA NAT Result is Positive

Category	HBV NAT Result†	HBsAg Result	Donor and Unit
1	Positive	Repeat Reactive / Confirmed Positive*	Discard unit; Permanently defer donor Donor not eligible for reentry
2	Positive	Non-Reactive	Discard unit; Indefinitely defer donor; Donor may be eligible for reentry
3	Positive	Repeat Reactive / Not Confirmed	

† Using a screening test, as described in section IV.A.2.
* Using either an HBsAg neutralization test or an HBV NAT with a limited supplemental test indication, as described in section III, and section IV.A.3.

C. Requalification Methods for Donors on the Basis of HBV NAT and HBV Serologic Test Results on the Follow-Up Sample

For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT having a sensitivity of < 2 IU/mL at 95% detection rate.

1. Requalification of a Donor of Whole Blood or Blood Components for Transfusion and Source Leukocytes for Further Manufacture

   To reenter an indefinitely deferred donor of Whole Blood or blood components for transfusion, or Source Leukocytes for further manufacture, a new sample should be obtained from the donor at least 6 months after the collection of the sample that gave test results described in section IV. B.2.c. (no donation is made at this time). You should perform follow-up testing using HBV NAT (having a sensitivity of ≤ 2 IU/mL at 95% detection rate), HBsAg and anti-HBc FDA-licensed assays.

   a. If the new follow-up sample tests positive by HBV NAT, regardless of HBsAg and anti-HBc test results, we recommend that you permanently defer the donor (Table 3, Category 1).

   b. If the new follow-up sample tests negative by HBV NAT and NR by HBsAg and anti-HBc assays, the donor may be reentered (i.e., the donor is eligible to donate in the future), provided the donor meets all donor eligibility criteria in § 640.3 (Table 3, Category 2).

   c. If the new follow-up sample tests negative by HBV NAT and RR by HBsAg and/or RR by anti-HBc, we recommend that you evaluate the

donor further as described in the FDA guidance documents cited in Refs. 1, 2 and 3 (Table 3, Category 3).

NOTE: If you wish to perform follow-up testing on a donor of Whole Blood or blood components for transfusion or a donor of Source Leukocytes for further manufacture who is deferred because of HBV NAT test results, you may do so before the end of the 6-month waiting period for donor notification purposes or for medical reasons. Negative test results on follow-up for HBsAg, anti-HBc and HBV DNA by NAT (sensitivity at 95% detection rate of ≤ 2 IU/mL), may be useful in donor counseling. However, only negative results for all three tests (HBsAg, anti-HBc and HBV NAT), obtained at least 6 months after the collection of the sample that gave test results described in section IV.B.2.c, would qualify the donor for reentry. If you obtain a reactive HBV NAT, or repeatedly reactive anti-HBc, or repeatedly reactive HBsAg that is positive by neutralization during this 6-month waiting period, the donor would not be eligible for reentry, and we recommend that you defer the donor permanently.

A donor of Whole Blood or blood components for transfusion, or a donor of Source Leukocytes for further manufacture who has been requalified as described above in section IV.C.1., may on subsequent occasions be indefinitely deferred because of HBV NAT reactive results. You may reenter such a donor into the donor pool by again following all the procedures described in section IV.C.1.

2. Requalification of a Donor of Source Plasma for Further Manufacture

To reenter an indefinitely deferred donor of Source Plasma, you should obtain a follow-up sample from the donor (no donation is made at this time) at least 6 months after the collection of the sample that gave the test results described in section IV.B.2.f. You should perform follow-up testing using HBV NAT (having a sensitivity of ≤ 2 IU/mL at 95% detection rate) and HBsAg FDA-licensed assays.

a. If a new follow-up sample tests positive by HBV NAT, regardless of the HBsAg test result, you should permanently defer the donor (Table 3, Category 1).

b. If a new follow-up sample tests negative by HBV NAT and NR by HBsAg, the donor is eligible to donate in the future, provided the donor satisfies all donor eligibility criteria in § 640.63 (Table 3, Category 2).

c. If a new follow-up sample tests negative by HBV NAT and RR HBsAg, you should evaluate the donor further, as described in the FDA documents cited in Ref. 1 (Table 3, Category 3).

NOTE: If you wish to perform follow-up testing on a donor of Source Plasma who is deferred because of HBV NAT test results, you may do so before the end of the 6-month waiting period for donor notification purposes or for medical reasons. Negative test results on follow-up for HBsAg and HBV DNA by NAT (sensitivity at 95% detection rate of ≤ 2 IU/mL), may be useful in donor counseling. However, only negative results for both tests (HBsAg and HBV NAT), obtained at least 6 months after the collection of the sample that gave the test results described in section IV.B.2.f, would qualify the donor for reentry. If you obtain a reactive HBV NAT, or repeatedly reactive HBsAg that is positive by neutralization, the donor would not be eligible for reentry, and we recommend that you defer the donor permanently.

A donor of Source Plasma who has been requalified as described above in section IV.C.2., may on subsequent occasions be indefinitely deferred because of HBV NAT positive results. You may reenter such a donor into the donor pool by again following all procedures described in section IV.C.2.

Table 3. Reentry of Donors of Whole Blood and Blood Components for Transfusion or Further Manufacture on the Basis of HBV NAT and HBV Serologic Test Results on the Follow-Up Sample

For purposes of reentry, we recommend that you use an FDA-licensed HBV NAT labeled as having a sensitivity of ≤ 2 IU/mL at 95% detection rate.

Category	HBV NAT Result (sensitivity of ≤ 2 IU/mL at 95% detection rate)	HBsAg and/or Anti-HBc Result (Anti-HBc not required for SP)	Donor
1	Positive	Any test result	Permanently defer donor
2	Negative	Non-Reactive	Donor may be eligible for reentry
3	Negative	Repeat Reactive	For further evaluation, see FDA guidance documents that discuss donor testing for HBsAg and anti-HBc. Refs. 1, 2 and 3.)

3. Management of Donors and Units with Non-Discriminated Reactive Test Results

If you obtain a reactive Multiplex HIV-1 RNA/HCV RNA/HBV DNA NAT result on an individual donor sample (ID-NAT), and if the Discriminatory NATs are non-reactive for HIV-1 RNA, HCV RNA and HBV DNA, the sample is "Non-Discriminated Reactive." The unit must be quarantined and destroyed (§ 610.40(h)), or, if released for research or further manufacture, be appropriately relabeled as described in section IV.C. The donor must be deferred (§ 610.41). Note that the donor should be deferred for 6 months and is eligible for reentry after the 6-month waiting period. If you choose to reenter the donor, you may do so at the time of a donation without prior testing of a follow-up sample.

V. LABELING

A. Circular of Information for Whole Blood and Blood Components Intended for Transfusion

Consistent with other donor screening tests, the instruction circular, also known as the "Circular of Information", must be updated to state that an FDA-licensed NAT for HBV DNA was used to screen donors and that the results of testing were negative (§ 606.122(h)). We recommend that you use the following statement on the labeling for donations that test Non-Reactive:

> "Licensed nucleic acid test (NAT) for HBV DNA has been performed and found to be Non-Reactive."

B. Blood Components Intended for Further Manufacture

Upon implementation of an FDA-licensed NAT, we recommend that you use the following statement on the labeling for blood components intended for further manufacture into injectable or non-injectable products that test Non-Reactive:

> "Non-Reactive for HBV DNA."

See paragraph C of this section for recommendations for donations that test Reactive for HBV.

C. Reactive Units and Product Disposition

NAT reactive units must not be shipped or used, except as provided in § 610.40(h)(2). If released for these uses, the units must be relabeled consistent with the labeling requirements in §§ 606.121, 610.40 and 640.70. Thus, for example, you must label the reactive unit with the "BIOHAZARD" legend and with the following cautionary statements, as applicable:

"Reactive for HBV DNA,"

and

"Caution: For Further Manufacturing into In Vitro Diagnostic Reagents For Which There Are No Alternative Sources."

In addition, you should label the reactive unit with the following legend, if applicable:

"Caution: For Laboratory Research Use Only."

VI. REPORTING CHANGES TO AN APPROVED APPLICATION

Under 21 CFR 601.12 (§ 601.12), FDA-licensed blood establishments are required to report changes to an approved biologics license application to FDA. FDA-licensed blood establishments must report the changes in paragraphs A, B, and C.1 and C.2.a of this section, as described below. However, except as specified in paragraph C.2.b of this section, unlicensed blood establishments are not required to report the changes to FDA.

A. Test Implementation

1. If you begin using an FDA-licensed NAT for the detection of HBV DNA in your facility according to the manufacturer's instructions, you must notify FDA of the testing change in your annual report (AR), in accordance with § 601.12(d), indicating the date that the revised standard operating procedures were implemented.

2. If you are already approved to use a registered contract donor testing laboratory to perform infectious disease testing of Whole Blood and blood components, including Source Plasma and Source Leukocytes, and the contract testing laboratory will now perform a NAT for HBV DNA, you must report this change in your AR (§ 601.12(d)).

3. If you will use a new contract testing laboratory to perform a NAT for HBV DNA, report as follows:

   a. If the new testing laboratory is registered with FDA and has been performing infectious disease testing for Whole Blood and blood components, including Source Plasma and Source Leukocytes, report this as a Changes Being Effected (CBE) Supplement, in accordance with § 601.12(c)(5).

   b. If the new testing laboratory has not previously performed infectious disease testing for blood products, you must report this as a Prior Approval Supplement (PAS), in accordance with § 601.12(b). The

new testing laboratory must register with FDA in accordance with 21 CFR Part 607 and § 610.40(f).

B. Labeling

Labeling refers to the instruction circular (e.g., Circular of Information) required under § 606.122 and the container labels on blood or blood components required under, among other provisions, §§ 606.121, 610.40 and 640.70.

1. If you revise your labeling to include the statements in this guidance in their entirety and without modification, you must report this change as a CBE labeling supplement in accordance with § 601.12(f)(2)).

2. If you revise your labeling to include alternative statements, you must report this change as a PAS labeling supplement in accordance with § 601.12(f)(1).

C. Procedures for Requalification of Donors

1. We consider the implementation of recommendations in this guidance in their entirety and without modification to be a minor change to an approved license application. Therefore, FDA-licensed establishments are not required to have FDA prior approval and may submit a statement of this change in their AR under § 601.12(d), indicating the date that the revised standard operating procedures were implemented.

2. Under § 610.41(b), you may only re-enter a previously deferred donor using a requalification method found acceptable by FDA for such purposes. We consider the requalification methods described in this guidance to be acceptable. If you choose to use an alternative requalification method, you must report this as follows:

   a. FDA-licensed blood establishments must submit the alternative requalification method as a PAS (§ 601.12(b)).

   b. Unlicensed blood establishments must submit the alternative requalification method to FDA before it is implemented so that we may determine whether it is acceptable.

VII. REFERENCES

1. FDA Memorandum to All Registered Blood Establishments: Recommendations for the Management of Donor and Units that are Initially Reactive for Hepatitis B Surface Antigen (HBsAg), December 2, 1987. http://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/otherrecommendationsformanufacturers/memorandumtobloodestablishments/default.htm.

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研究報告の概要

○日本における年齢及び地域別有病率によるC型肝炎、B型肝炎の診断未確定キャリア総数の推定
目的: 日本でC型肝炎ウイルス(HCV)、B型肝炎ウイルス(HBV)の診断未確定キャリアの総数を推定すること。
方法: 地域及び年齢別のHCVならびにHBV有病率は、20-39歳については初回供血者(n=2,429,364)、40-74歳については定期健康診断の受診者(HCV;n=6,204,968、HBV;n=6,228,967)にて調査された。5-19歳の若者の有病率は一つの県において決定され(HCV;n=79,256、HBV;n=68,792)、75歳以上の高齢者のHCV有病率は指数モデルにて推定された。HBV感染はHBs抗原の検出により決定し、HCV感染はアルゴリズムまたは抗HCV抗体を持つ個人の70%が持続性感染であると仮定する事のどちらかにより決定した。
結果: 2005年の127,285,653人の全人口のうち、807,903人(95%CI; 679,886-974,292)がHCVキャリアであると算出され(0.63%)、903,145人(95%CI; 837,189-969,572)がHBVキャリアであると算出された(0.71%)。C型肝炎の年齢による特徴は、年齢とともに有病率の変動幅が大きくなることである。地域別の特徴は、有病率の変動幅が大きく異なり、西日本で増幅が大きい傾向があることである。一方B型肝炎では、全地域において55～59歳で最も有病率が高く、北海道では3.1%に上った。どの地域においても2000年と2005年を比較すると、HCV及びHBVキャリアの割合は減少している。
結論: 一般集団におけるHCV及びHBVの診断未確定感染者の正確な評価は、肝疾患の発症の可能性を予測し、健康管理の改善に対する適切な対策に寄与する。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
日本におけるHCV、HBVキャリアの総数は、全人口のうちHCVは約81万人(0.63%)、HBVは約90万人(0.71%)であると算出されたとの報告である。	HBV、HCV感染に関する新たな知見等について、今後も情報の収集に努める。

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Total Numbers of Undiagnosed Carriers of Hepatitis C and B Viruses in Japan Estimated by Age- and Area-Specific Prevalence on the National Scale

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Key Words
Hepatitis C virus · Hepatitis B virus · Blood donors · Liver cirrhosis · Hepatocellular carcinoma · Healthcare · Japan

Abstract
Objective: To estimate total numbers of undiagnosed carriers of hepatitis C virus (HCV) and hepatitis B virus (HBV) in Japan. ***Methods:*** Area- and age-specific prevalence of HCV as well as HBV was determined in the first-time blood donors [20–39 years (n = 2,429,364)] and examinees of periodical health check-ups [40–74 years (6,204,968 for HCV and 6,228,967 for HBV)] in Japan. Prevalence in adolescents [5–19 years (79,256 for HCV and 68,792 for HBV)] was determined in a single prefecture, and that of HCV in the elderly (≥75 years) was estimated by the exponential model. HBV infection was determined by the detection of hepatitis B surface antigen, and HCV infection by either the algorithm or assuming persistent infection in 70% of the individuals with antibody to HCV. ***Results:*** Of the total population of 127,285,653 in 2005, 807,903 (95% CI 679,886–974,292) were estimated to be infected with HCV at a carrier rate of 0.63%, and 903,145 (837,189–969,572) with HBV at that of 0.71%. ***Conclusion:*** Accurate estimation of undiagnosed HCV and HBV carriers in the general population would help to predict the future burden of liver disease, and take appropriate measures for improving healthcare.

Introduction

Hepatitis C virus (HCV) and hepatitis B virus (HBV) are estimated to infect 170 and 350 million people over the world, respectively [1, 2]. Most infections with HCV or HBV do not induce clinical liver disease, while ~30% of them develop severe liver disease such as cirrhosis and hepatocellular carcinoma [3, 4]. Hence, there is a pressing need to identify the individuals who have undiagnosed HCV or HBV infection, and take effective measures for terminating viral infections and preventing the progression of liver disease.

For management of persistent HCV and HBV infections in a given country, it is necessary to know their exact numbers for assessing medical and financial needs in the foreseeable future. Prevalence of undiagnosed HCV or HBV

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infection has been estimated by survey of blood donors in Japan [5] and a representative population in the USA [6].

In the present study, area- and age-specific prevalence of HCV or HBV infection was determined in 8 jurisdiction areas of the Japanese Red Cross Blood Center. Then, the total numbers of undiagnosed HBV and HCV infections were estimated by compiling the results in the first-time blood donors and examinees of the periodical health check-up program. Of the 127,285,653 Japanese registered in 2005, 807,903 (0.63%) were estimated to be infected with HCV and 903,145 (0.71%) with HBV.

Materials and Methods

Japanese Population

Japan is divided into 8 areas, along its north-to-south axis, according to jurisdiction of the Japanese Red Cross Blood Center, i Hokkaido, Tohoku, Kanto, Hokuriku/Tokai, Kinki, Chu- Shikoku and Kyushu. Populations in 5-year age groups in jurisdiction area were obtained from the registry at the National Census 2005.

First-Time Blood Donors

During 6 years from January 2001 to December 2006, 3,748,422 individuals (aged 16–64 years) donated whole blood or apheresis products for the first time, and their sera were tested for markers of HCV and HBV infections. Ongoing HCV infection was estimated by assuming the detection of HCV RNA in 70% of individuals with the antibody to HCV (anti-HCV), in accordance with a previous report [5].

Examinees of Hepatitis Virus Infections

Since the fiscal year 2002 in Japan, individuals who turned 40, 45, 50, 55, 60, 65 and 70 years were offered to take tests for hepatitis viruses at periodical health check-ups by a 5-year national project. During 5 years through 2006, 6,204,968 individuals received tests for HCV and 6,228,967 for HBV, corresponding to ~30% of the eligible Japanese, and their area- and age-specific prevalence of HCV or HBV infection was determined.

School Children and Adolescents

In the Iwate prefecture located in the north of Japan, biochemical markers of diseases dependent on the lifestyle were examined in children and adolescents at the entrance to schools. Their serum samples had been stored frozen, and were tested for markers of hepatitis virus infections. Carrier rates of HCV and HBV among them were calculated, with their ages adjusted to those in 2005; infants aged <5 were represented by the children aged from 5 to 9 years. Designs and procedures of this investigation were approved by the Ethics Committee of Hiroshima University.

Simulation of HCV and HBV Infections in the Elderly

By its age-specific profile, the prevalence of HCV was deduced to be an exponential function of the age. Accordingly, age-specific prevalence of HCV in the individuals aged ≥75 years was simulated by an exponential function model; it was constructed on the prevalence of HCV in each age group ≥50 years.

The formula was constructed as:

$$\log y(x) = a + bx$$

where x is the 5-year age code, $y(x)$ is an estimator of HCV prevalence in x, and a and b are coefficients.

The equation is transformed into:

$$y(x) = e^a e^{bx}$$

in which e^a represents the HCV prevalence when $x = 0$ (in the group aged 0–4 years), since $y(0)$ is equal to e^a. By replacing x for $x + 1$ in the above equation, it is converted to $y(x + 1) = e^a e^{b(x+1)}$.

Then, the following equation can be constructed:

$$y(x + 1) = e^b y(x)$$

where e^b is the slope of HCV prevalence increasing with age. Thus, the HCV prevalence is multiplied by a factor e^b for an increment of the age code by 1.

The simulation model was applied to estimate age-specific prevalence of HCV in each of 8 areas in the individuals ≥75 years.

Prevalence of HBV in the individuals ≥75 years was represented by that in those aged 70–74 years, since it stayed constant from 65 through 75 years.

Markers of Hepatitis Virus Infections

In blood donors, anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories, North Chicago, Ill., USA) with a cutoff limit set at 2^5, as well as by particle agglutination with commercial assay kits (HCV PA Test-II; Fujirebio, Inc., Tokyo, Japan). HBsAg was determined by reversed passive hemagglutination with reagents prepared by the Japanese Red Cross.

In examinees of periodical health check-ups, ongoing HCV infection was determined by the algorithm with anti-HCV and HCV RNA [7]. Anti-HCV was determined by passive hemagglutination of the second generation with commercial assay kits (HCV PHA; Abbott Laboratories), and since 2002, it was determined by enzyme immunoassay with commercial assay kits (AxSYM HCV Dinapack-III; Abbott Laboratories). Samples with high anti-HCV titers contain HCV RNA, and therefore, only those with low and middle titers were examined for HCV RNA. HBsAg was determined by reversed-passive hemagglutination with commercial assay kits (Institute of Immunology Co., Ltd, Tokyo, Japan).

Statistical Analyses

Statistical analyses for the evaluation of R^2 values were performed with JMP 8.0 (SAS Institute, Inc., Cary, N.C., USA) and DeltaGraph 5.5 (RedRock Software, Inc., Salt Lake City, Utah, USA). A p value > 0.05 was considered significant.

Results

Age-Specific Prevalence of HCV in the First-Time Blood Donors and Examinees of Periodical Health Check-Ups

Figure 1 illustrates age-specific prevalence of HCV in the first-time blood donors (aged 15–69 years in 2005) and examinees of periodical health check-ups (39–73 years in 2005); 70% of individuals with anti-HCV were considered

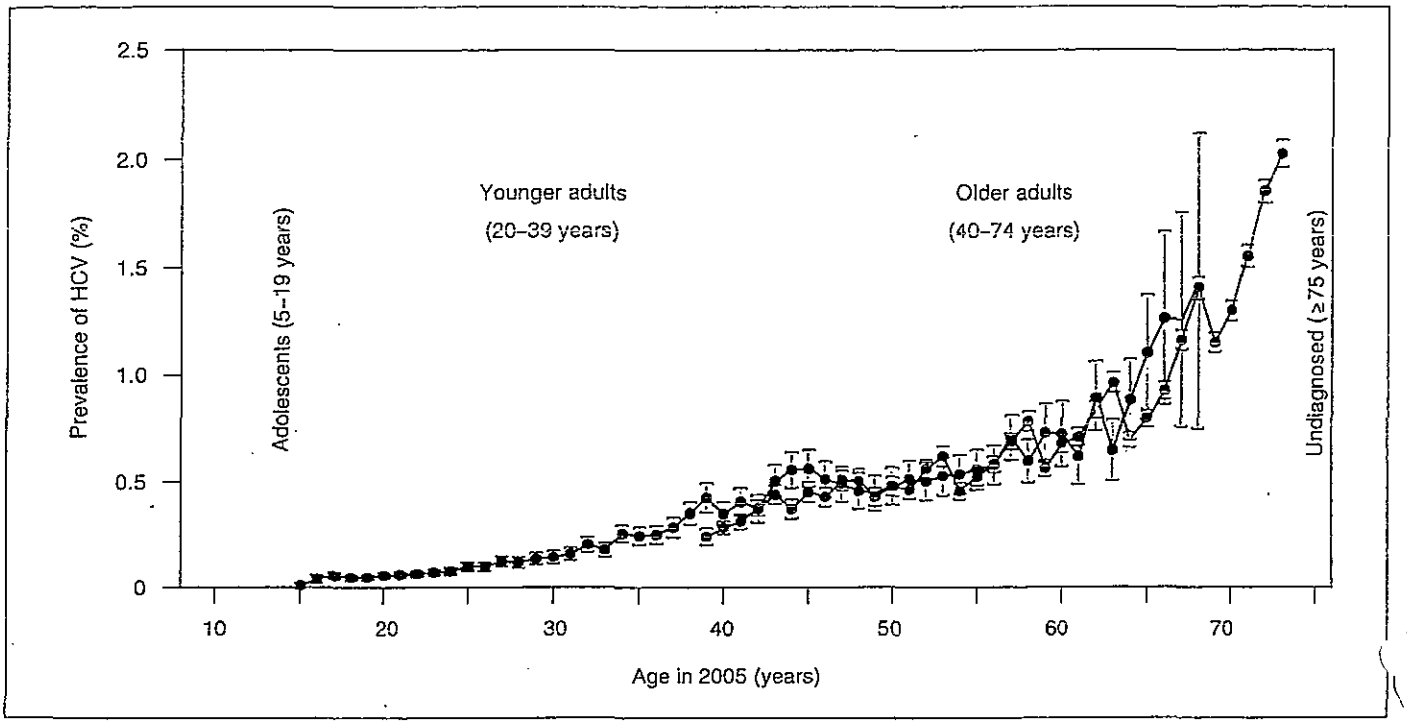

Fig. 1. Age-specific prevalence of HCV in Japan. The prevalence of HCV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) and examinees of periodical health check-ups aged from 39 to 73 years (red dots). Their ages were adjusted to those in the year 2005. Bars indicate ranges of 95% CI.

to possess HCV RNA in serum [5]. Results of two distinct populations were well in accord. For the first-time blood donors, however, the variation (95% CI) widened increasingly with age. It would have reflected decreases in the first-time blood donors with age, since the majority of these (83.5%) were aged ≤39 years. As the prevalence of HCV in blood donors ≥40 years was unreliable in them, that in examinees of periodical check-ups was adopted for estimating the national prevalence of HCV.

Area-Specific Prevalence of HCV in Eight Jurisdiction Areas

In view of distinct geographic distribution of HCV, the prevalence of HCV in the general population would not be applicable to every area in Japan. Figure 2 compares results in the first-time blood donors and recipients of health check-ups among 8 jurisdiction areas spanning from north (Hokkaido) to south (Kyushu). They unfolded a wide variety in the age-specific prevalence of HCV. Although the prevalence of HCV increased with age in all areas, the slope of increase differed widely among them. Hence, it was necessary to employ a distinct age-specific prevalence in each of the 8 areas for estimating HCV carriers precisely.

Table 1. Age-specific prevalence of HCV in three different populations

Age in 2005	n	HCV-positive, n	Prevalence, % (95% CI)
School children			
5–9	17,390	2	0.012 (0.000–0.027)
10–14	29,817	3	0.010 (0.000–0.021)
15–19	32,049	7	0.022 (0.006–0.038)
Blood donors			
20–24	1,205,966	1,122	0.065 (0.061–0.070)[a]
25–29	536,560	874	0.114 (0.105–0.123)[a]
30–34	408,814	1,089	0.186 (0.173–0.200)[a]
35–39	278,024	1,190	0.300 (0.279–0.320)[a]
HCV screening			
40–44	611,146	2,127	0.348 (0.333–0.363)
45–49	495,032	2,292	0.463 (0.444–0.482)
50–54	675,350	3,485	0.516 (0.499–0.533)
55–59	947,438	5,974	0.631 (0.615–0.646)
60–64	1,081,854	8,423	0.779 (0.762–0.795)
65–69	1,264,496	13,722	1.085 (1.067–1.103)
70–74	1,054,472	17,649	1.674 (1.649–1.698)

[a] The prevalence in blood donors was based on an assumption of HCV infection persisting in 70% of those with anti-HCV [5].

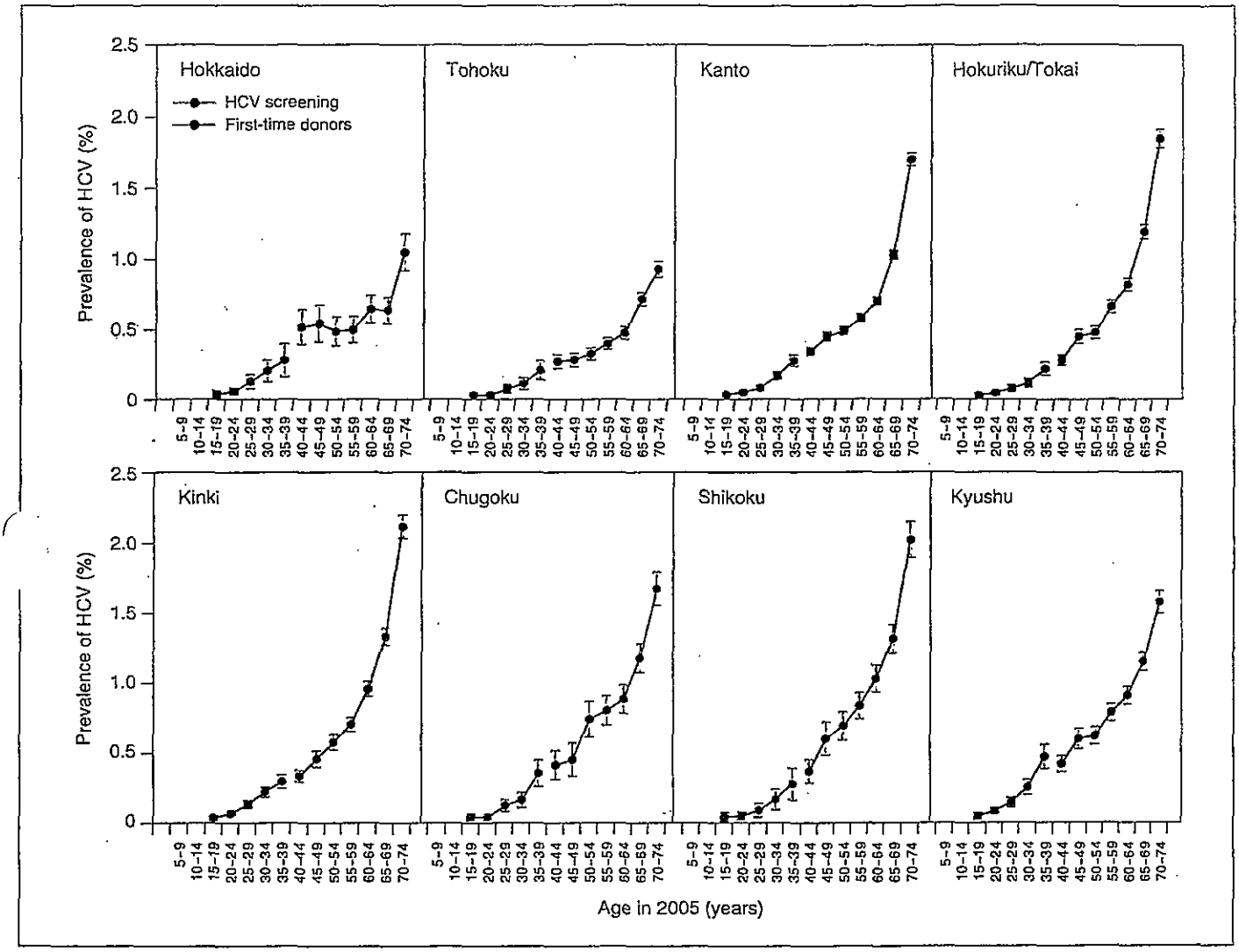

Fig. 2. Age-specific prevalence of HCV in 8 jurisdiction areas in Japan. The prevalence of HCV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors, and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate *ranges of 95% CI.*

Prevalence of HCV in Adolescents

Since blood donors were restricted to 16–64 years of age, and health examinees were targeted on 40–70 years, they did not cover individuals aged ≤15 or ≥75 years in the year 2005. To fill in an opening on the younger side, the age-specific prevalence of HCV was determined in school children and adolescents in the Iwate prefecture (table 1). The prevalence in infants aged 0–4 years was assumed similar to that in the children aged 5–9 years; an extremely low prevalence of HCV (0.012%) would support such an assumption.

Simulating Prevalence of HCV in the Elderly

The prevalence of HCV appeared to be an exponential function of the age, according to its profiles in the first-time blood donors and examinees of health check-ups (fig. 1). Based on this assumption, a formula was constructed to simulate the prevalence of HCV in age groups ≥75 years for each of the 8 jurisdiction areas in Japan (see Materials and Methods).

Figure 3 compares actual (dots) and simulated data (red line) of five age groups from 50 to 74 years (corresponding to age codes 10–14) among the 8 areas. There was a high coefficient of determination between them,

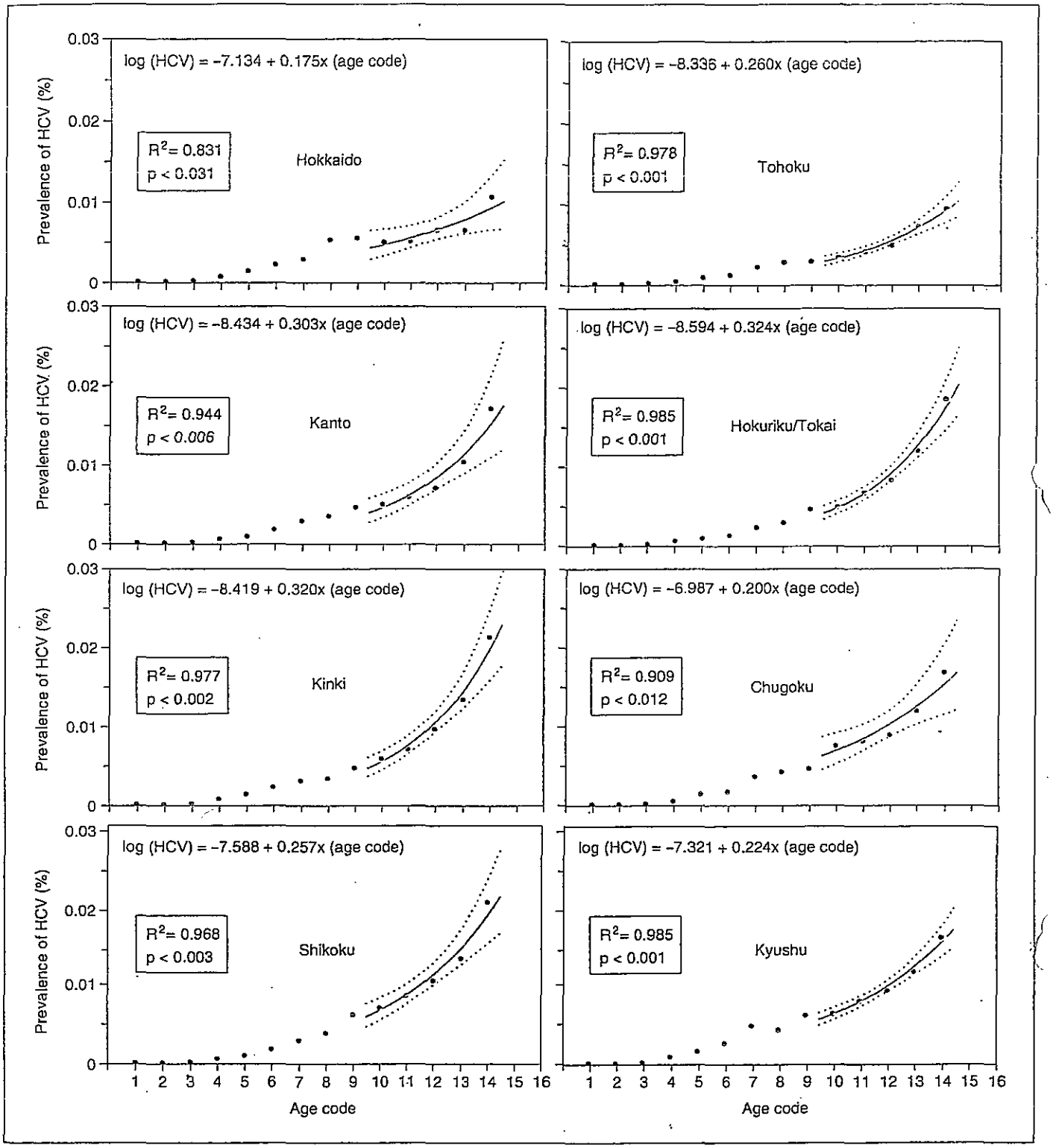

Fig. 3. Simulation of age-specific prevalence of HCV in the elderly. Prevalence of HCV in the first-time blood donors as well as examinees of periodical health check-ups (dots) and that simulated by formulation (red line with ranges of 95% CI in dotted line) are shown for 8 jurisdiction areas in Japan. Formula is shown at the top of each area. Age codes are: 1, 5–9 years; 2, 10–14 years; 3, 15–19 years; 4, 20–24 years; 5, 25–29 years; 6, 30–34 years; 7, 35–39 years; 8, 40–44 years; 9, 45–49 years; 10, 50–54 years; 11, 55–59 years; 12, 60–64 years; 13, 65–69 years; 14, 70–74 years, and 15, 75–79 years.

Table 2. Regional and total HCV carriers in Japan

Areas	Population	HCV carriers (95% CI)	Carrier rate
Hokkaido	5,620,813	26,097 (19,356–34,413)	0.46%
Tohoku	12,047,975	50,688 (42,754–59,953)	0.40%
Kanto	41,247,892	235,328 (195,408–293,611)	0.57%
Hokuriku/Tokai	19,294,443	132,434 (114,216–154,446)	0.69%
Kinki	22,657,542	173,808 (147,548–207,173)	0.52%
Chugoku	7,650,977	53,296 (42,299–67,698)	0.70%
Shikoku	4,083,698	35,159 (28,746–43,004)	0.86%
Kyushu	14,682,313	101,092 (89,379–113,993)	0.80%
Total	127,285,653	807,903 (679,886–974,292)	0.63%

Table 3. Age-specific prevalence of HBV in three different populations

Age in 2005	n	HBV-positive, n	Prevalence, % (95% CI)
School children			
5–9	17,363	3	0.017 (0.000–0.037)
10–14	29,817	14	0.047 (0.022–0.072)
15–19	32,049	12	0.037 (0.016–0.059)
Blood donors			
20–24	1,205,966	1,826	0.151 (0.144–0.158)
25–29	536,560	1,650	0.308 (0.293–0.322)
30–34	408,814	1,759	0.430 (0.410–0.450)
35–39	278,024	1,327	0.477 (0.452–0.503)
HBV screening			
40–44	613,960	5,491	0.894 (0.871–0.918)
45–49	497,589	5,373	1.080 (1.051–1.109)
50–54	679,893	8,700	1.280 (1.253–1.306)
55–59	950,508	12,891	1.356 (1.333–1.379)
60–64	1,085,119	13,282	1.224 (1.203–1.245)
65–69	1,268,304	12,406	0.978 (0.961–0.995)
70–74	1,057,469	9,545	0.903 (0.885–0.921)

with R^2 values ranging from 0.831 to 0.985 ($p < 0.031$ and $p < 0.001$, respectively), attesting to the validity of this simulation. Of note, the factor *b* in formula (by which age codes were multiplied) varied broadly among the 8 areas. Thus, it was the highest in Hokuriku/Tokai at 0.324 and lowest in Hokkaido at 0.175, with close to twofold differences between them.

Estimation of Undiagnosed HCV Carriers in Eight Areas and the Entire Nation

Based on age- and area-specific prevalence of HCV, numbers of undiagnosed HCV carriers were calculated for 8 jurisdiction areas, and they were compiled in the entire nation (table 2). The prevalence of HCV in each of three age groups (75–79, 80–84 and ≥85 years) was simulated by the formula, while that of HBV was represented by the prevalence in the group of 70–74 years. As of the year 2005, 127,285,653 were registered in the national census of Japan, and 807,903 of these are estimated to have undiagnosed HCV infection at an overall carrier rate of 0.63%. There was an increasing gradient in the prevalence of HCV along the north-to-south axis of Japan.

Age-Specific Prevalence of HBV

Figure 4 depicts age-specific prevalence of HBV in 2005. It was deduced from HBsAg in the first-time blood donors (15–69 years) and examinees of periodical health check-ups (39–73 years). Since the prevalence of HBV in the elderly did not increase with age so sharply as that of HCV (fig. 1), it was presumed not to increase further and stay around 1% in the individuals ≥75 years. The age-specific prevalence of HBV tabulated in three different

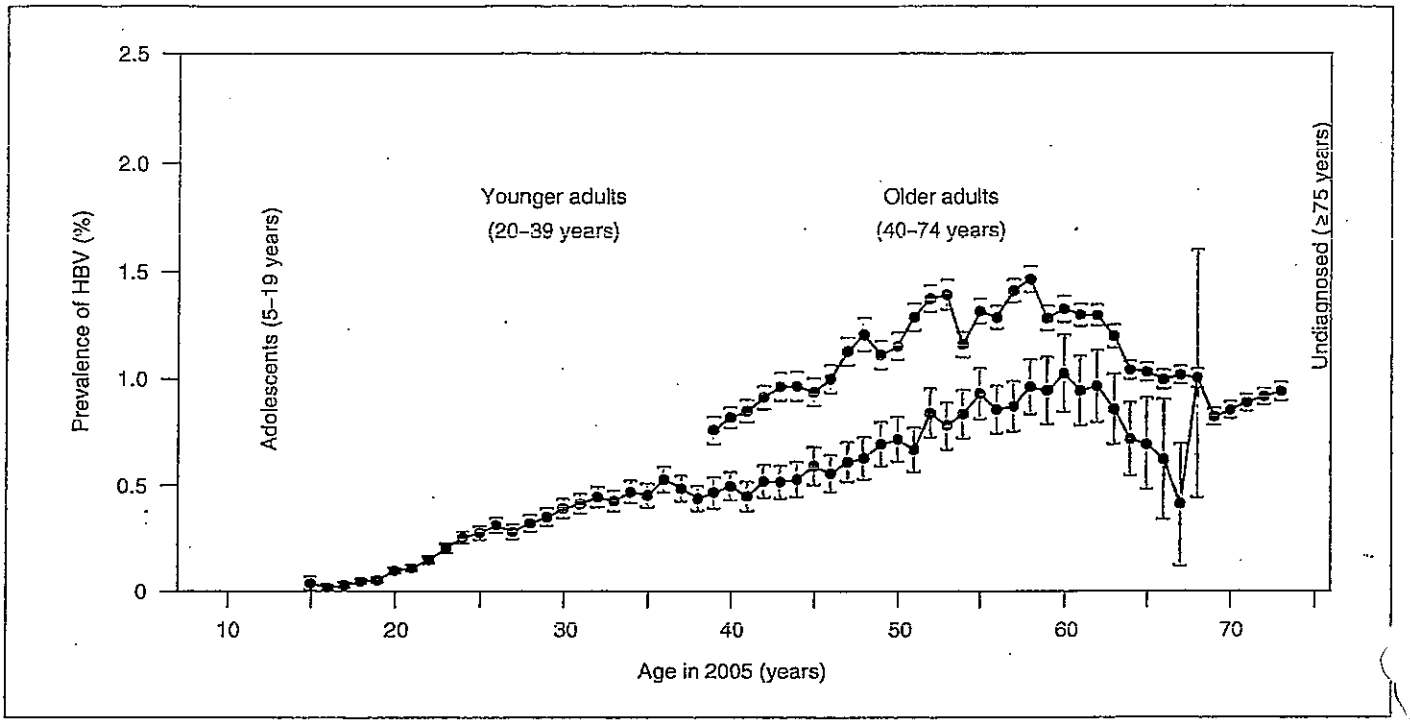

Fig. 4. Age-specific prevalence of HBV in Japan during 2002–2006. The prevalence of HBV was determined in the first-time blood donors aged from 15 to 68 years (blue dots) in the year 2005 and examinees of periodical health check-ups aged from 39 to 73 years (red dots) in the year 2005. Bars indicate ranges of 95% CI.

populations is listed in table 3. There was a constant decline with decreasing age in the frequency of HBV in individuals ≤39 years, and it was particularly low in children ≤9 years (0.017%).

In examinees of periodical health check-ups, the age-specific prevalence of HBV did not diverge and stayed within a narrow 95% CI (fig. 4). By contrast, that in the first-time blood donors dispersed widely. Such a variation in the age-specific prevalence of HBV would have been ascribed to the first-time blood donors who clustered in age groups ≤40 years.

Area-Specific Prevalence of HBV in Eight Jurisdiction Areas

The age-specific prevalence of HBsAg varied widely among 8 jurisdiction areas (fig. 5). HBsAg was most frequent in the age group of 55–59 years in every area, and reached 3.1% in the northern-most Hokkaido. The peak frequency decreased in central Japan (1.1% in Kanto and Hokuriku/Tokai), and increased towards the southern end (1.9% in Kyushu). Thus, the prevalence of HBsAg was determined individually along the axis of Japan in estimating the total number of HBV carriers in Japan.

Estimation of Undiagnosed HBV Carriers in Eight Areas and the Entire Nation

Numbers of undiagnosed HBV carriers were complied by multiplying age-specific prevalence of HBsAg by corresponding subpopulations in 8 jurisdiction areas (table 4). In total, 903,145 of the 127,285,653 (0.71%) individuals are estimated to have undiagnosed HBV infection in Japan in 2005.

Shift of Undiagnosed HCV and HBV Carriers during 5 Years (2000–2005) in Japan

Table 5 compares numbers of HCV and HBV carriers aged 15–69 years between 2000 and 2005 for 8 jurisdiction areas in Japan. Data for the year 2000 were extracted from a previous survey [5]. Data for the year 2005 were obtained in the first-time blood donors during 2001–2006 in this study by the same method as in the previous survey [5]. Undiagnosed HCV and HBV carriers decreased during 5 years by 55 and 47.5%, respectively. The overall carrier rate of HCV declined sharply from 0.95 to 0.44%, and that of HBV from 1.04 to 0.55% in Japan.

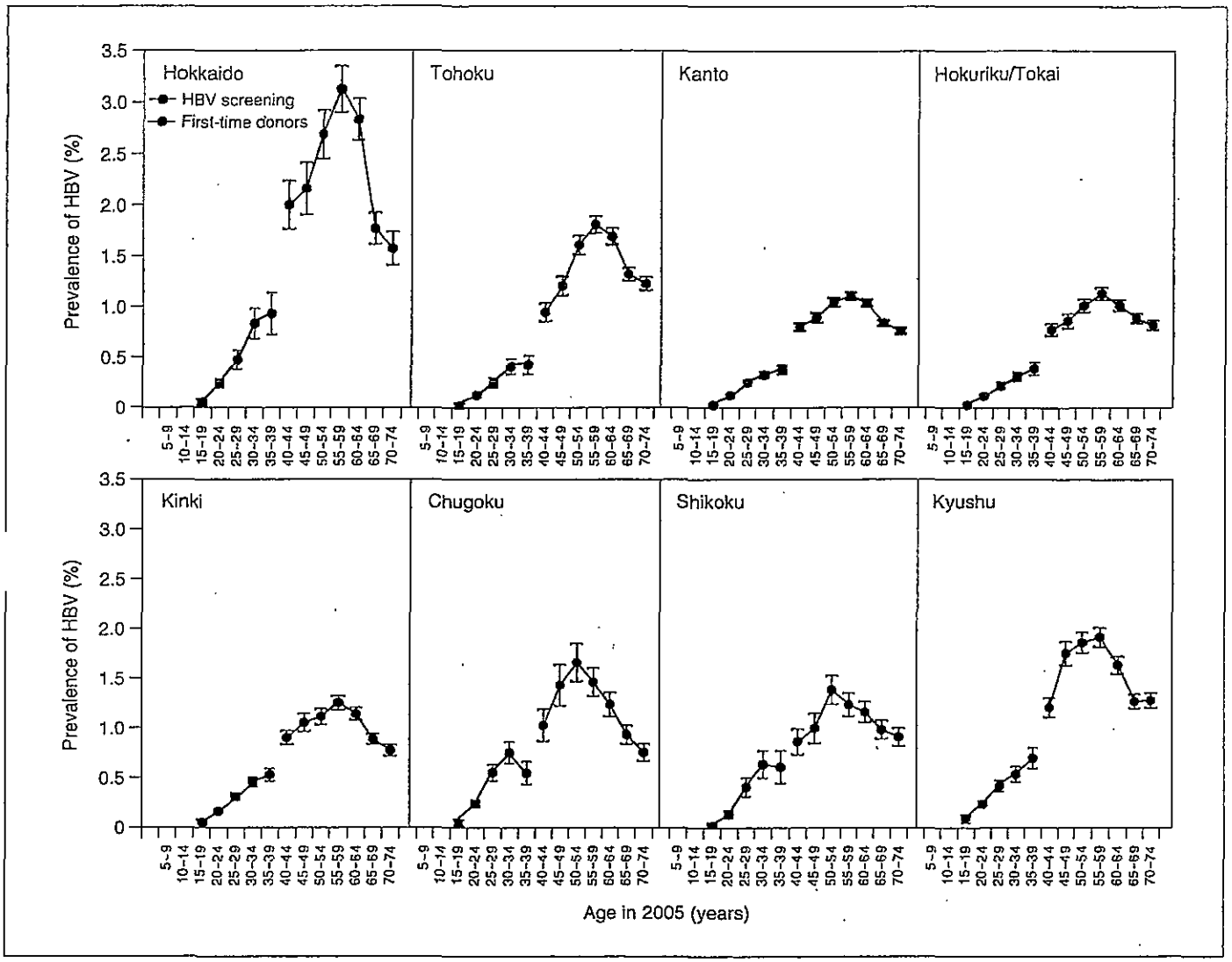

Fig. 5. Age-specific prevalence of HBV in 8 jurisdiction areas in Japan. The prevalence of HBV is calculated in each of twelve age groups notched by 5 years. The prevalence in five groups ≤39 years was represented by the first-time blood donors and that in seven groups ≥40 years by recipients of HCV screening. Bars indicate ranges of 95% CI.

Table 4. Regional and total HBV carriers in Japan

Areas	Population	HBV carriers (95% CI)	Carrier rate
Hokkaido	5,620,813	80,573 (72,314–88,765)	1.43%
Tohoku	12,047,975	104,736 (97,742–111,816)	0.87%
Kanto	41,247,892	231,799 (220,129–244,105)	0.56%
Hokuriku/Tokai	19,294,443	109,709 (101,722–117,581)	0.56%
Kinki	22,657,542	144,965 (134,387–155,464)	0.64%
Chugoku	7,650,977	59,948 (52,705–67,121)	0.78%
Shikoku	4,083,698	29,776 (26,080–33,437)	0.73%
Kyushu	14,682,313	141,639 (132,111–151,282)	0.96%
Total	127,285,653	903,145 (837,189–969,572)	0.71%

Table 5. Decrease of undiagnosed HCV and HBV carriers in the 15- to 69-year-old population in Japan

	Survey in 2000[a]		Survey in 2005		Difference	
	number estimated	carrier rate in area[b]	number estimated	carrier rate in area[b]	number estimated	balance
Shift of HCV carriers during 5 years from 2000 to 2005						
Hokkaido	41,139	0.99%	17,658	0.44%	−23,481	−57.1%
Tohoku	61,658	0.71%	30,525	0.37%	−31,133	−50.5%
Kanto	277,644	0.90%	126,283	0.41%	−151,361	−54.5%
Hokuriku/Tokai	88,724	0.64%	48,360	0.35%	−40,364	−45.5%
Kinki	178,871	1.06%	70,526	0.43%	−108,345	−60.6%
Chugoku	72,431	1.32%	24,595	0.47%	−47,836	−66.0%
Shikoku	43,497	1.49%	16,504	0.59%	−26,993	−62.1%
Kyushu	120,989	1.16%	64,115	0.63%	−56,874	−47.0%
Total	884,954	0.95%	398,567	0.44%	−486,387	−55.0%
Shift of HBV carriers during 5 years from 2000 to 2005						
Hokkaido	106,896	2.56%	54,557	1.35%	−52,339	−49.0%
Tohoku	104,923	1.21%	48,490	0.58%	−56,433	−53.8%
Kanto	255,207	0.83%	132,414	0.43%	−122,793	−48.1%
Hokuriku/Tokai	78,481	0.56%	51,477	0.37%	−27,004	−34.4%
Kinki	165,915	0.98%	85,083	0.52%	−80,832	−48.7%
Chugoku	90,041	1.64%	37,706	0.71%	−52,335	−58.1%
Shikoku	38,411	1.32%	19,162	0.69%	−19,249	−50.1%
Kyushu	127,879	1.23%	77,941	0.77%	−49,938	−39.1%
Total	967,753	1.04%	506,830	0.55%	−460,923	−47.6%

[a] Data for the year 2000 were extracted from a previous survey of hepatitis virus infections in Japan [5].
[b] The carrier rate specific for respective jurisdiction area was applied.

Discussion

There are many constraints in estimating total HCV and HBV infections in a given nation. Since it is not feasible to test every member for serological markers of hepatitis virus infection, populations representative of the entire nation have served for the estimation. Volunteer blood donors are recruited, but they have a restricted age range (16–64 years in Japan). Students attending schools and universities can close the opening in younger generations, but infants younger than the school age are not enrolled. Moreover, there are no means of estimating carrier rates of hepatitis virus infections in the individuals aged beyond the eligibility of blood donation. In addition, blood donors are selected individuals who are leading healthy lives above the average. In the survey of inhabitants in sentinel counties of the USA [6], who represent the average Americans, patients with liver disease and persons with restricted activities, such as those incarcerated or institutionalized, are not included.

Patients with clinical liver disease, as well as individuals found with HCV or HBV infection by health check-ups, can receive the medical care. However, many blood donors found with viral infections have developed severe liver disease already, and therefore, cannot receive efficient medical interventions [7, 8]. Hence, it is necessary to detect undiagnosed HCV and HBV infections hidden in the society. For this purpose, periodical health check-ups for screening hepatitis virus markers were started in April 2002 on the individuals, who turned 40, 45, 50, 55, 60 and 70 years, by a 5-year national project in Japan. The target age range (40–70 years) was selected due to a high incidence of hepatocellular carcinoma [9]. Since by far the majority of the first-time blood donors were younger than 40 years, the prevalence of HCV or HBV beyond that age dispersed widely (fig. 1, 4). In this study, therefore, the coverage by the first-time blood donors was confined to 20–39 years of age, and it was taken place by examinees of health check-ups aged 40–74 years; they left age groups ≤15 and ≥75 years uncovered, however.

The national prevalence of hepatitis virus infections in individuals ≤19 years was presumed to be similar to that in the Iwate prefecture situated in northern Japan. Since the prevalence of HCV or HBV infection in them was extremely low and stayed between 0.01 and 0.02%, such an assumption would not have affected the overall results to any significant extent. The prevalence of HCV in age groups ≥75 was simulated by a premise that it would be an exponential function of the age. Consequently, the formula based on profiles in five age groups from 50 to 74 years (at a 5-year notch) was extrapolated to three age groups ≥75 years. The simulation matched closely with the prevalence determined in corresponding age groups, with R^2 values ranging from 0.83 to 0.99 ($p < 0.05$ and $p < 0.01$, respectively) throughout 8 jurisdiction areas in Japan (fig. 3).

Japan has an axis spanning 2,000 kilometers from the north-east towards the south-west over the four major lands (Hokkaido, Honshu, Shikoku and Kyushu). Within a rather small land, the prevalence of HCV or HBV is not uniform all over Japan. The prevalence of HCV had an increasing gradient from north to south, and was the highest in Kyushu (table 2), while that of HBV was the highest in Hokkaido, decreased in between and then increased towards Kyushu (table 4). Reflecting such local differences, age-specific prevalence of HCV or HBV differed widely among 8 jurisdiction areas (fig. 2, 5).

Based on the results obtained on the area- and age-specific prevalence of HCV or HBV, carriers of these hepatitis viruses in 8 jurisdiction areas were tabulated separately over age groups from 20 to 74 years. Those in age groups ≤19 years were represented by the Iwate prefecture. The prevalence of HCV in age groups ≥75 years was simulated by the formula, and that of HBV was represented by individuals aged 70–74 years. Japan was populated by 127,767,994 people in 2005. Of these, 807,903 (95% CI 679,886–974,292) were estimated to have undiagnosed HCV infection at an overall prevalence of 0.63%, and 903,145 (837,189–969,572) to possess undiagnosed HBV infection at that of 0.71%. These estimates are much less than publically inferred numbers of HCV and HBV carriers in Japan at 1.5–2.0 million each. Leaving aside HCV and HBV carriers who have developed liver disease and stayed outside the scope of the present study, our estimates based on reasonable scientific grounds are much smaller; they add up barely half of generally referred figures around 1.5–2.0 million in Japan.

Based on the sex- and age-specific prevalence of hepatitis virus markers in the 3,478,422 first-time blood donors during 2001–2006, with the same criteria used in the previous study [5], we have estimated the number of undiagnosed HCV carriers aged 15–69 years in the year 2005 to be 398,567 (95% CI 295,410–501,453) and that of undiagnosed HBV carriers to be 506,830 (95% CI 398,115–616,113). In the previous study [5], undiagnosed HCV and HBV carriers aged 15–69 years in the year 2000 were assessed to be 884,954 (95% CI 725,082–1,044,826) and those with HBV to be 967,753 (95% CI 806,760–1,128,745). They decreased by 55.0 and 47.6%, respectively, during 5 years (table 5). In support of this view, the incidence of HCV or HBV infection during 10 years (1994–2000) in Japan is very low and estimated at 1.86 (95% CI 1.06–3.01) or 2.78 (1.87–4.145) per 100,000 person-years [10]. Decreases in undiagnosed HCV and HBV carriers in Japan would have been attributed to increased chances of receiving tests for hepatitis virus infections at health checkups and medical institutions, as well as increased awareness due to educational programs or other healthcare campaigns or screening programs in high-risk individuals. Additionally, there would have been a cohort effect in individuals aged 15–69 years who have shifted by 5 years during the observation period.

The results of the Third National Health and Nutrition Survey (HANES III, 1988–1994) [11] and those of more recent HANES (2001–2002) [6] in the USA are essentially similar with respect to age-specific profiles of HCV infection, and shifted by 10 years. The incidence of de novo HCV and HBV infections may have decreased substantially both in the USA and Japan, driven partly by the introduction of the nucleic acid amplification test and a more stringent questionnaire on donors to exclude blood donations in the window period of infection [12–17]. The national burden of HCV infection has been reported in Great Britain [18], where the prevalence of anti-HCV in hospitalized patients was 3.4% and that in the first-time blood donors was 0.03% in the year 2008.

In spite of many improvements in the control of hepatitis virus infections, there are many HCV and HBV carriers buried in the society who need immediate identification for receiving timely and efficient medical interventions. Treatment of viral hepatitis keeps improving, especially for liver disease induced by HCV. The sustained virological response in the patients infected with HCV of genotype 1, who have received triple therapy with pegylated interferon, ribavirin and protease inhibitors, has increased to 70% or higher, from 50% with the state-of-care therapy with pegylated interferon and ribavirin [19, 20]. With the advent of new antiviral drugs that will enter the scene in the foreseeable future, the virological response is expected to increase further. There would be

nothing like early detection of HCV and HBV infections for appropriate and timely medical care to prevent the progression of liver disease. Such a rational strategy will benefit not only patients themselves, but also merit the society and government, which are going to be burdened with ever-increasing morbidity and mortality along with skyrocketing costs.

Acknowledgements

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医薬品
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識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	厚生労働省処理欄
				2011年8月10日	該当なし	

一般的名称	①②乾燥抗HBs人免疫グロブリン ③ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	Vox Sanguinis 2011;:1-2	公表国 アメリカ,欧州,東南アジア
販売名（企業名）	①ヘブスブリン筋注用200単位　（ベネシス） ②ヘブスブリン筋注用1000単位　（ベネシス） ③ヘブスブリンIH静注1000単位　（ベネシス）			

研究報告の概要

我々は血漿由来医薬品の製造に使われる血漿分画プールのHEV RNAと抗HEV IgGを調査した。血漿プール(n=75)は欧州、北米、中東及びアジアから入手した。プールの約10%はHEV RNA陽性で、広範囲な地理的分布を示した（陽性プール：北米(n=1)、西欧(n=2)、東欧(n=1)、アジア(n=4)）。中東からのプールでは陽性サンプルは見つからなかった。陽性プールの何れもHEV RNA量が＞1000コピー/mLを超えていなかった。プールで見つかった低ウイルス量は、最初のクリオプレシピテートの後、更なる処理工程が行われる血漿由来凝固因子の何れの最終製剤からも、なぜMordow他がHEV RNAを検出しなかったかを説明するだろう。
別の可能性のある要因は、研究所間で大きく異なることが分かっているHEV RNA NAT分析法の感度であり、これは現在標準化されていない。献血者のHEVウイルス血症は10^7HEV RNAコピー/mLを超える可能性がある、従って、製造時に使われるプール・サイズとウイルス除去工程次第であるが、幾つかの血漿分画製剤でHEV RNAを検出できるかもしれない可能性はある。
プールで確認されたHEV株の系統樹解析（GenBank、受入番号：JN257704－JN257711）は、欧州と北米のプールで遺伝子型3ウイルスが見つかり、一方アジアのプールは地域で多くみられる遺伝子型4ウイルスを含んでいた。全配列は全てが他と異なっていた。プールで確認された2つの遺伝子型は、ヒトと共にブタのような動物の両方で見つかり、それは幾つかの症例における人獣感染の可能性を示唆した。プール中の抗HEV IgGの存在は、MP BiomedicalsとAxiomの酵素免疫測定法を使って測定した。アジアのプールだけがMP Biomedicalsの測定系のカットーオフより大きい抗HEV IgG量を持っていることが分かった、そして結果はAxiomキットを使って確かめられ、地域の抗HEV抗体陽性率を反映した。抗HEVが幾つかのプールで特定されたものの、これが潜在的ウイルスの感染能の中和と関連するのかについては未だ明らかではない。最近の研究では、抗HEVを含むウイルス血症血清を使った培養で感染性HEVを増殖させることが可能であった。HEVは輸血によって伝播したが、プールした血漿によるHEV伝播の報告はない。HEVはナノろ過（＜20nm）によって除去できる、そしてそれはおそらく加熱処理によって不活化されると思われるが、その効果は不活化の条件や組成に依存している。しかし、抗HEV抗体量が低い地域では、HEVなどのノン・エンベロープウイルスに対する有効な除去工程を有さないSD処理血漿については、血漿プールのHEV RNAを検査するのが賢明かもしれない。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

報告企業の意見	今後の対応
HEVは直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

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LETTER

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Vox Sanguinis

Widespread distribution of hepatitis E virus in plasma fractionation pools

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Dear Editor,

We have investigated plasma fractionation pools, used in the manufacture of plasma-derived medicinal products, for HEV RNA and anti-HEV IgG. Plasma pools were obtained from Europe, North America, the Middle East and Asia. Approximately 10% of pools were positive for HEV RNA, showing a widespread geographic distribution (Table 1). Positive pools originated from plasma sourced in North America (n = 1), Western Europe (n = 2) and Eastern Europe (n = 1). No positive samples were identified in the pools from the Middle East. Of the Asian pools, four were positive for HEV RNA. None of the positive pools exceeded a load of >1000 copies/ml HEV RNA. The low viral loads found in pools may explain why Mordow *et al.* [1] did not detect HEV RNA in any final preparations of plasma-derived coagulation factors, which undergo further processing steps after initial cryoprecipitation. Another possible factor is the sensitivity of HEV RNA NAT assays that have been shown to vary widely between laboratories [2] and is not currently standardized. HEV viraemia in blood donors can exceed 7 $\log_{10}$ HEV RNA copies/ml [3]; therefore, dependent on pool size and virus reduction steps used during manufacture, it is possible that in HEV RNA might still be detectable in some plasma derivatives.

Phylogenetic analysis of the HEV strains identified in the pools (GenBank, accession numbers JN257704–JN257711) revealed that genotype 3 viruses were found in Europe and North America, whilst the Asian pools contained genotype 4 viruses that are more common in the region. All sequences were distinct from one other. The two genotypes identified in pools are found both in humans as well as in animal such as swine, with likely zoonotic transmission in some cases.

The presence of anti-HEV IgG in pools was determined using enzyme immunoassays from *MP* Biomedicals (*MP* Biomedicals Asia Pacific, Singapore) and Axiom (Axiom Diagnostic, Bürstadt, Germany). Only Asian pools were found to have anti-HEV IgG levels greater than the cut-off of the MP Biomedicals assay, and results were confirmed using the Axiom kit, reflecting the seroprevalence of anti-HEV in the region. Whilst anti-HEV is detectable in some pools, how this correlates to neutralization of potential virus infectivity remains unknown. In a recent study, it was possible to propagate infectious HEV in culture using viraemic serum containing anti-HEV [4]. There are no reports of HEV transmission by pooled plasma, although HEV has been transmitted by transfusion. HEV can be removed by nanofiltration (<20 nm), and it may be inactivated by heat treatment, the effectiveness of which is dependent upon parameters for inactivation and composition of the matrix [5]. However, where anti-HEV levels are low, solvent/detergent-treated plasma, with no effective reduction steps against non-enveloped viruses such as HEV, could present a risk for transmission, and it may be prudent to test such plasma pools for HEV RNA.

Table 1 Analysis of plasma fractionation pools for the presence of HEV RNA

Source of pools	No. positive/ no. analysed
Europe	3/34
Europe/North America	0/3
North America	1/4
Middle East	0/11
Southeast Asia	4/23
Overall	8/75

RT-PCR was performed using total nucleic acid extracted using the COBAS AmpliPrep instrument (Roche Diagnostics GmbH, Penzberg, Germany). HEV RNA was detected using the One-Step RT-PCR kit (Qiagen GmbH, Hilden, Germany) using conserved primers (forward 5′ GGG TGG AAT GAA TAA CAT GT and reverse 5′ AGG GGT TGG TTG GAT GAA) amplifying 233-bp products that were analysed by agarose gel electrophoresis and sequenced directly for genotyping purposes.

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医薬品
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識別番号・報告回数			報告日	第一報入手日 2011 年 10 月 5 日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	Vox Sanguinis 2011; Article published on line 29 SEP	公表国 イギリス
販売名 （企業名）	①テタノブリン IH 静注 250 単位　（ベネシス） ②テタノブリン IH 静注 1500 単位　（ベネシス） ③テタノブリン筋注用 250 単位　（ベネシス）			

研究報告の概要

英国における献血者のミニプール血漿中のE型肝炎ウイルス RNA の検出

我々は（世界中の幾つかの地域から始まった）血漿分画プール中のE型肝炎ウイルス(HEV) RNAと抗体の検出を報告したベイリー等からの手紙を興味深く読んだ。著者等はプールの10%がHEV RNA陽性であった、そして血漿由来医薬品を使うことによる感染リスクを論ずるために報告した。
我々は最近、供血者パネル及び輸血に関連した伝播の可能性があるウイルス転換を示す英国とウェールズの献血者の現在のHEV感染の証拠を報告した。英国の血液供給への更なるHEVのリスクを把握するために、血清学的及び分子調査が2007年に集められた血漿ミニプールで行なわれた。それぞれのミニプールは48人の供血者から構成され、本来C型肝炎RNAスクリーニング様に準備したものだった。HEV RNAの抽出と検出は、前述した様に880のミニプール（約42,000人の供血者に相当）で実施された。880プール中の6つ(0.7%)がHEV RNAを検出可能であった。予想通り、HEV RNA陽性プール中のウイルス量は低かった（≦2000GEq以上/mL）。追加のHEV抗体（抗HEV）検査は、6つ全て(100%)で見つかった、そしてHEV RNA陽性プールの1/6(17%)はHEV IgGとIgMにそれぞれ反応した。検査した100のHEV RNA陰性プールの内、73%がHEV IgGに反応、HEV IgMは0%であった。
HEVによる無症候性感染の高い発生率は、献血者が受血者を感染させるに十分な機会を与える。一般的な英国人で行なわれた調査は13%以上の抗HEV血清陽性率を示し、年間6万人以上の症例が発生すると推定する。従って、我々の調査は検査したミニプール中の高い抗HEV IgG陽性率が示されたことに恐らく驚いていない。HEV RNAと抗HEV IgMの検出は、現在のHEV感染を示している。対照的に、英国の血清陽性率が与えた大変な驚きとして、ベイリー等はアジアからのプールだけでHEV IgGを発見した。彼らはまた、ヨーロッパからの検査したプール中のHEV RNAが8倍以上の高率であったことを報告したが、プールサイズを開示していない。これらの異いの幾つかは、プールの構成及び使われた検出分析のバラツキよって説明されるかもしれない。
まとめると、これらの報告は血液/血液成分と血液製剤からHEVを伝播する可能性の証拠を提供した。しかしながら、輸血後HEV伝播の範囲及びHEVを含んだ輸血製剤の受け入れの結果は、不十分な調査されていない。輸血関連のHEVリスクは免疫抑制した持続性HEVの著しい危害に関する新たなデータに照らして考慮に値する。英国の血液/血液成分の75%以上は、この集団に血液学的な支持として与えると推定する。従って、HEVと血液の安全性の課題は、更なる調査と議論が必要である。

報告企業の意見	今後の対応
HEVは直径27～38nmの球状粒子で、エンベロープはなく、長さ約7,300塩基対の一本鎖RNAを内包している。万一、原料血漿にHEVが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスクリアランス試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・
その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
2. 重要な基本的注意
1) 本剤の原材料となる血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の破傷風抗毒素を含有する血漿を原料として、Cohn の低温エタノール分画で得た画分からポリエチレングリコール 4000 処理、DEAE セファデックス処理等により抗破傷風人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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LETTER

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Vox Sanguinis

Detection of hepatitis E virus RNA in plasma mini-pools from blood donors in England

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Dear Editor,

We read with interest the letter from Baylis *et al.*, [1] reporting on the detection of hepatitis E virus (HEV) RNA and antibody in plasma fractionation pools, which originated from several regions across the globe. The authors report that 10% of the pools were HEV RNA positive and discuss the transmission risk through the use of plasma-derived medicinal products.

We recently reported evidence of current HEV infection in English and Welsh blood donors indicating a turnover of the virus in the donor panel and the potential for transfusion-associated transmission [2]. To ascertain further the risk of HEV to the English blood supply, serological and molecular investigations were undertaken in plasma mini-pools collected in 2007. Each mini-pool was made up of 48 individual donors and had originally been prepared for hepatitis C RNA screening. Extraction and detection of HEV RNA was carried out on 880 mini-pools (equivalent to approximately 42 000 individual donors) as previously described [2]. Six of the 880 pools (0·7%) had detectable HEV RNA. As expected, viral loads in the HEV RNA-positive pools were low (≤ 2000 GEq/ml). Additional HEV antibody (anti-HEV) testing found all 6 (100%) and 1/6 (17%) of the HEV RNA-positive pools to be anti-HEV IgG and IgM reactive respectively. Of the 100 HEV RNA-negative pools tested, 73% and 0% were HEV IgG and IgM reactive respectively.

The high incidence of asymptomatic infection with HEV gives ample opportunity for blood donors to infect recipients. Studies undertaken in the general English population indicate an anti-HEV seroprevalence of ~13% and estimate that ~60 000 cases occur per year [3]. It is therefore perhaps unsurprising that our study demonstrates a high anti-HEV IgG prevalence in the mini-pools tested. The detection of HEV RNA and anti-HEV IgM demonstrates current HEV infections. In contrast, Baylis *et al.* [1] found HEV IgG only in the pools from Asia, which is very surprising given the UK seroprevalence. They also report ~eightfold higher rates of HEV RNA in tested pools from Europe but do not disclose the pool size. Some of these differences may be explained by variations in the make up of the pools and in the detection assays used.

Collectively, these reports provide evidence of the potential to transmit HEV from blood/blood components and products. However, the extent of HEV transmission post-transfusion and the outcome of receiving HEV-containing transfusion products remain poorly explored. The risks of transfusion-associated HEV deserves due consideration in light of emerging data on the significant harm of persistent HEV in the immunosuppressed [4, 5]. It is estimated that ~75% of UK blood/blood components are given as haematological support to this population. The issue of HEV and blood safety therefore warrants further studies and debate.

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識別番号・報告回数			報告日	第一報入手日 2011 年 10 月 3 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	Transfusion (Transfusion) 2011; 51(9): 1896-1908	公表国 アメリカ	
販売名 (企業名)	①テタノブリン IH 静注 250 単位　(ベネシス) ②テタノブリン IH 静注 1500 単位　(ベネシス) ③テタノブリン筋注用 250 単位　(ベネシス)					

研究報告の概要

パルボウイルスB19(B19V)に対するレセプターが赤血球上にあるため、我々は試験管内のスパイク実験で血中のB19ウイルスの分布を調査し、自然感染におけるウイルスのコンパートメント化と持続性を評価した。
二つの全血プロトコル（超遠心分離法のプロトコルと急速赤血球溶解/除去のプロトコル）は、定量リアルタイム・ポリメラーゼ連鎖反応を用いて評価した。全血に既知濃度のB19ウイルスがスパイクされ、様々な血液分画中における回収率が測定された。それから、急速赤血球溶解/除去のプロトコルが、「REDS 同種供血者と受血者の貯蔵庫（RADAR）」の凍結検体の中の43名のB19ウイルス感染ドナーから長期的に集められた104対の全血と血漿のペアにおけるB19ウイルス濃度の比較に使用された。
B19ウイルススパイク実験では、ウイルスDNAの約3分の1は血漿中で回収され、3分の2は赤血球に緩く結合していた。血漿B19ウイルスDNA濃度が100IU/mL以上の献血者の免疫グロブリン(Ig)M陽性期において、DNA濃度の中央値は血漿中よりも全血中で約30倍高かった。対照的に、IgM陰性時やB19ウイルスDNA濃度が低い時、全血対血漿比の中央値は約1であった。長期間の検体の分析は、全血中のB19ウイルスは持続的に検出されるが、血漿に対する全血のB19ウイルスの比は、血漿におけるウイルス量減少とIgM反応性低下を伴って減少することを明らかにした。
血漿に対する全血の B19 ウイルス DNA 比は感染ステージにより変化する：IgM 陽性期では、全血では血漿の 30 倍の B19 ウイルス DNA 濃度があり、その後 IgG 抗体のみが存在する持続感染期にはその比は同等になる。このことが、B19 ウイルスに感染した赤芽球由来の DNA 陽性赤血球の存在と関係するか、B19 ウイルスに特異的な IgM 抗体を介したウイルスの細胞への結合と関係するかを決定するため、更なる研究が必要がある。

使用上の注意記載状況・
その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
1. 慎重投与
(1)略
(2)略
(3)溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(4)免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
6. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎

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報告企業の意見	今後の対応	
ヒトパルボウイルスB19（human parvovirus B19：B19）は、脂質エンベロープを持たない極めて小さな（約20～26nm）DNAウイルスで、輸血や血漿分画製剤による伝播が報告されている。他のウイルスに比べて、血漿分画製剤の製造工程での不活化・除去が困難であり、本ウイルスの伝播リスクを完全に否定することはできないため、1996年11月より、使用上の注意にB19についての記載を行い注意喚起を図ってきた。万一、原料血漿にB19が混入したとしても、CPVをモデルウイルスとしたウイルスクリアランス試験成績及びB19を用いた不活化･除去試験の結果から、本剤の製造工程において十分に不活化・除去されると考えている。なお、原料血漿へのB19混入量低減のため、B19-ミニプールNATが米国の原料供給元で行われている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。	児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

Distribution of parvovirus B19 DNA in blood compartments and persistence of virus in blood donors

Tzong-Hae Lee, Steven H. Kleinman, Li Wen, Lani Montalvo, Deborah S. Todd, David J. Wright, Leslie H. Tobler, and Michael P. Busch for the NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II)

BACKGROUND: Because the receptor for parvovirus B19 (B19V) is on red blood cells (RBCs), we investigated B19V distribution in blood by in vitro spiking experiments and evaluated viral compartmentalization and persistence in natural infection.

STUDY DESIGN AND METHODS: Two whole blood (WB) protocols (ultracentrifugation and a rapid RBC lysis and removal protocol) were evaluated using quantitative real-time polymerase chain reaction. WB was spiked with known concentrations of B19V and recovery in various blood fractions was determined. The rapid RBC lysis and removal protocol was then used to compare B19V concentrations in 104 paired WB and plasma samples collected longitudinally from 43 B19V-infected donors with frozen specimens in the REDS Allogeneic Donor and Recipient Repository (RADAR).

RESULTS: In B19V spiking experiments, approximately one-third of viral DNA was recovered in plasma and two-thirds was loosely bound to RBCs. In the immunoglobulin (Ig)M-positive stage of infection in blood donors when plasma B19V DNA concentrations were greater than 100 IU/mL, median DNA concentrations were approximately 30-fold higher in WB than in plasma. In contrast, when IgM was absent and when the B19V DNA concentration was lower, the median WB-to-plasma ratio was approximately 1. Analysis of longitudinal samples demonstrated persistent detection of B19V in WB but declining ratios of WB to plasma B19V with declining plasma viral load levels and loss of IgM reactivity.

CONCLUSIONS: The WB-to-plasma B19V DNA ratio varies by stage of infection, with 30-fold higher concentrations of B19V DNA in WB relative to plasma during the IgM-positive stage of infection followed by comparable levels during persistent infection when only IgG is present. Further study is required to determine if this is related to the presence of circulating DNA-positive RBCs derived from B19V-infected erythroblasts, B19V-specific IgM-mediated binding of virus to cells, or other factors.

The understanding of the natural history of parvovirus B19 virus (B19V) infection is that in most immunocompetent individuals (e.g., blood donors), viremia occurs approximately 1 week after infection and persists at high titers in plasma for approximately 5 days.[1] Immunoglobulin (Ig)M antibody develops at approximately 12 days after infection and IgG antibody follows within days coinciding with precipitous declines in plasma viremia levels. Subsequently, plasma viremia disappears generally within weeks, IgM antibody becomes undetectable after several months (although this precise duration is unknown), whereas IgG antibody persists long term and is thought to convey immunity to reinfection. Recently it has become established that a variation of this natural history occurs in people in whom chronic persistent B19V infection occurs; this is characterized by low plasma levels of B19V DNA persisting for more than 6 months in conjunction with IgG antibody.[2-7]

The receptor for B19V on marrow red blood cell (RBC) progenitor cells is the P blood group antigen.[8,9] This receptor is also present at high concentrations on mature

ABBREVIATIONS: B19V = parvovirus B19; CBER = Center of Biologics Evaluation and Research; RADAR = REDS Allogeneic Donor and Recipient Repository; TC = target capture; VL(s) = viral load(s); WB = whole blood.

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circulating RBCs in almost all individuals, with the exception of rare persons with the null p phenotype. Binding of B19V to mature RBCs is known to occur and has been exploited in development of RBC B19V antigen agglutination assays.[10] Thus, it is theoretically possible that a substantial proportion of B19V in blood is adsorbed to or persists within RBCs from the infected erythroblast phase and that B19V DNA concentrations will consequently differ in plasma and cellular blood compartments. Also, it is unknown if the partitioning of B19V between plasma and cellular blood compartments varies during different stages of infection, possibly due to the effect of IgM and IgG antibodies on B19V particles enhancing or blocking binding to one or more cellular blood elements (e.g., RBCs, white blood cells [WBCs], or platelets [PLTs]).

B19V contamination of plasma derivatives has led to widespread adoption of B19V DNA screening of source and recovered plasma donations to interdict high-titer viremic units before pooling and fractionation.[11-13] Transfusion-transmitted B19V infection from blood component transfusion occurs infrequently but has been documented in several case reports, including a recent case in the United States.[14] Although screening of whole blood (WB) components intended for individual patient transfusions is not currently routinely performed (except in Germany, Austria, and Japan, where this screening is performed on plasma and targets units with high B19V DNA concentration), the issue of compartmentalization of B19V in blood could be important if policies evolve toward further testing.

This study's major objective was to establish the relative concentrations of B19V DNA in plasma versus WB and to determine if this "compartmentalization" varies in different stages of infection. To accomplish this, we developed procedures to apply a sensitive B19V polymerase chain reaction (PCR) assay to WB samples. This involved a series of in vitro spiking experiments to establish that 1) B19 viral standards contained intact viral particles that could be pelleted by our ultracentrifugation protocol; 2) spiking high-titer B19V standards into fresh and frozen WB to establish the partitioning of exogenously spiked B19V in various blood compartments; and 3) development of a special reagent to overcome the inability of the ultracentrifugation-target capture (TC) protocol (as demonstrated in our experiments) to reliably recover and detect low levels of B19V DNA in WB. After this in vitro experimental work, we then used our standard plasma TC-PCR and this novel WB protocol to test serial samples from donors with previously documented B19 plasma viremia whose samples were stored in the REDS Allogeneic Donor and Recipient Repository (RADAR). As a secondary objective, we also evaluated the rate of B19V DNA persistence in those B19V DNA–positive donors who had a repository donation that was given at least 6 months before or subsequent to their DNA-positive donation.

MATERIALS AND METHODS

Standard B19V assays

Plasma B19V PCR assay

This assay has previously been described in detail.[15,16] It is based on a magnetic-bead B19V DNA capture step followed by a TaqMan real-time PCR assay targeting the VP1 region of the Genotype 1 B19V genome. Assay sensitivity was established as a 50% limit of detection of 1.6 IU/mL (95% confidence interval [CI], 1.2-2.1) and a 95% limit of detection of 16.5 IU/mL (95% CI, 10.6-33.9). When run against a standard curve, the assay can also be used to quantify B19V DNA with the lower limit of quantitation at 20 IU/mL. Alternately, in in vitro spiking studies a difference in cycle threshold (i.e., the cycle at which DNA was initially detected) was used to compare relative quantities of B19V DNA in different blood compartments.

B19V antibody testing

Specimens were tested for the presence of B19V IgG and IgM antibodies against a recombinant VP2 protein with Food and Drug Administration (FDA)-licensed test kits (Biotrin, Dublin, Ireland). Due to sample volume considerations, testing was performed in singlicate (rather than in duplicate as stated in the package insert) by accessing a 0.25-mL subaliquot of plasma from the RADAR repository. If results fell into the equivocal zone, the assay was repeated in singlicate and the repeat result was taken as the overall final result for the specimen.[15,16]

Protocol for development of WB PCR assays and for in vitro spiking studies to compare levels of B19V in plasma versus WB compartments

B19V standard and spiked controls

We used a Genotype 1 B19V standard from the Center of Biologics Evaluation and Research (CBER, Rockville, MD) to prepare our spiking B19V preparation. The CBER *B-19–positive standard*[17] *was from a window period* plasma donation. We generated plasma and WB-spiked controls at serial twofold dilutions, with concentrations equal to 1000, 500, 250, 125, 62.5, and 31.25 IU/mL. These spiked standards were aliquoted and frozen at –80°C. Unspiked samples were also prepared as negative controls. These standards were used in both the ultracentrifugation and the WB processing (HemoBind, US patent application pending; http://www.faqs.org/patents/app/20100092980) protocols. To make a relevant and equitable comparison all the WB and plasma assays used in this study were designed to assay 0.5 mL per reaction, as was used in our previous study.

Validation of the ultracentrifugation protocol using plasma controls

Plasma spiking standards, as described above, were ultracentrifuged for 2 hours (4°C) at 50,377× *g* (Sorvall Stratos, Thermo Scientific, Asheville, NC) to pellet intact viral particles and thereby confirm that B19V DNA in the CBER standard was virion associated and that the ultracentrifugation protocol could efficiently recover all B19V in the standard. The supernatant was removed and the pellet digested with proteinase K (20 mg/mL) overnight; these samples were processed by B19V TC and amplified using our real-time PCR protocol. The results from the ultracentrifugation protocol were compared to the results generated using our standard assay procedure applied to corresponding spiked plasma preparations that were not subjected to ultracentrifugation and pellet extraction.

Ultracentrifugation protocol for frozen WB

Fresh WB, spiked with B19V to achieve a concentration of 100,000 IU/mL (derived from a high-titer plasma donation that tested negative for B19 IgM and IgG antibodies by the Biotrin assay), was incubated at room temperature for 1 hour and then aliquoted and frozen at –80°C. The frozen blood was thawed and mixed with an equal volume of RBC saponin lysis solution (0.4% saponin in 0.5% NaCl, pH 7.4) to completely lyse residual RBC in the already hemolyzed thawed-frozen WB. The preparation was ultracentrifuged at 50,377× *g* for 2 hours. The supernatant was transferred to a second tube, leaving the degraded RBC membranes and viral particles in a pellet in the primary tube. The pellet was subjected to protein digestion by adding 200 uL of an equal part of Solution A (0.1 M KCl, 0.01 M Tris Base pH 8.3, 0.0025 M $MgCl_26H_2O$) and Solution B (10 mM Tris, pH 8.3, 2.5 mM $MgCl_26H_2O$, 1% Tween-20, 1% NP40) mixed with 1.25 uL of proteinase K (20 mg/mL). Protein digestion was performed at 60°C, for 2 hours, vortexing vigorously every 15 minutes. Both pellets and supernatants were tested for B19V DNA by TC and real-time PCR amplification.

Separation of WB into compartments after incubation with B19V

Plasma containing B19 virions was spiked into freshly drawn WB to achieve a concentration of 100,000 IU/mL followed by incubation at room temperature for 24 hours. After incubation, the WB was centrifuged at 833 × *g* for 5 minutes to separate the plasma from the packed red cell (pRBC) compartment, which includes the buffy coat layer. The plasma collected at this point served as reference data for other compartments.

In a "without wash" experiment, after removal of plasma, the pRBCs were not washed and were directly subjected to RBC lysis followed by ultracentrifugation to generate a pellet composed of viral particles, RBC membranes, and WBCs/PLTs. In the "wash" experiment, after removal of plasma, the remaining pRBCs with buffy coat (WBCs/PLTs) were washed twice with PBS to ensure that viral particles that were not tightly bound to cells were washed away before proceeding with RBC lysis using a Saponin RBC lysis solution. After lysis, the preparation was centrifuged at 4000 × *g* for 5 minutes, generating a hemolyzed RBC supernatant and a pellet of WBCs and large PLTs. The supernatant, containing lysed RBC membranes and presumably bound viral particles, was collected into a tube and subsequently ultracentifuged. The pellet was washed again to ensure no residual lysis solution remained. The RBC supernatant and the wash solutions were ultracentrifuged at 50,377 × *g* (Sorvall Stratos, Thermo Scientific) for 2 hours to bring down viral particles, RBC membranes, and viral particles bound to the membranes. The pellet and the supernatant gathered after ultracentrifugation were collected for testing. The RBC membrane pellets from the ultracentrifugation and the WBC and PLT pellets were subjected to protein digestion. B19V DNA was extracted from all compartments using TC and amplified using real-time PCR.

Novel RBC lysis and removal protocol for WB preparations

To address the problem of processing WB for detection of both plasma and cell-associated nucleic acids without use of ultracentrifugation, we developed a new method for rapid RBC lysis and removal of hemoglobin (Hb) and other possible inhibitors of TC and PCR processing (HemoBind WB processing protocol). An equal volume of 6 molar guanidine-HCl-0.2 mol/L ethylenediaminetetraacetat lysis buffer (pH 8.0) was added to 0.5 mL of frozen-thawed WB. The sample was vortexed and incubated at room temperature while gently mixing in a rocker. The sample was pulse spun at high speed in a microcentrifuge to pellet particulate material; all cell- and plasma-derived nucleic acids would be expected to remain in the guanidine lysate supernatant. One milliliter of HemoBind buffer was added to 1 mL of the lysate supernatant, along with 20 µL of proteinase K (20 mg/mL). The mixture was vortexed and incubated at 60°C for 30 minutes, and then clarified of precipitates by centrifugation in a high-speed microcentrifuge for 1 minute. The supernatant was transferred to a clean mirocentrifuge tube and heated at 100°C for 5 minutes. The preparation was microcentrifuged at high speed for 1 minute and the clear supernatant was transferred to the 10-tube units used in the Procleix TC system. The protocol for plasma TC was followed. As with the plasma protocol, the WB samples were amplified with internal controls. Results from samples with positive internal controls were deemed valid. Valid results with less or equal to cycle threshold of 40 were considered positive and those with amplifications greater than cycle threshold of 40 were considered negative.

Selection of paired plasma and WB samples from the RADAR repository

The National Heart, Lung, and Blood Institute RADAR repository was established to investigate possible transfusion-transmitted infections and has been described in detail elsewhere.[18] Repository specimens were collected from 2000 through 2003 by blood centers and selected hospitals at seven geographically dispersed US locations. All enrolled subjects gave informed consent for frozen storage of their specimens and for subsequent testing of these specimens for possible transfusion-transmissible infections. There was no restriction on how many times a repeat donor could be recruited to give a repository sample.

The donor portion of the repository consists of 13,201 donation specimens (given by 12,408 distinct donors) that were transfused to enrolled recipients and 99,906 donation specimens (contributed by 84,339 donors) from donations that were not transfused to enrolled RADAR recipients. Repository specimens consisted of two frozen 1.8-mL plasma aliquots and a 1.5-mL sample of frozen WB.

In previous studies, B19V DNA and antibody testing had been performed on plasma samples from 17,549 donor repository samples (5020 from donors without enrolled recipients and 12,529 from donors with enrolled recipients).[15,16] As previously reported, this testing identified 149 samples from 147 donors that tested positive for B19V DNA in plasma. For this study, we searched through the RADAR repository records to determine whether any of the donors with positive PCR results had made additional repository donations which had not yet been evaluated for B19V DNA. We found that of the 149 PCR-positive donors, 43 had samples from more than one blood donation included in the repository. In total, there were 137 donations given by these 43 donors. The index samples (i.e., those previously selected for B19V analysis), the immediate previous and subsequent donations, and the first and last donation in donors with greater than three donations from each of these 43 donors were selected for this study; additional intervening donations were also tested in selected cases. Thus, a total of 104 donations were tested by plasma B19V PCR, WB B19V PCR using the HemoBind protocol, and B19V IgM and IgG antibody assays. Quantitative results from plasma and WB PCR assays were obtained as two separate replicate determinations with results reported as the mean of these results. Personnel performing the study testing were kept blinded as to the identities of the paired WB and plasma samples. For the 43 index donations, we used the plasma B19V PCR and antibody results from previous studies rather than performing these tests again due to limitations in sample volumes.

We constructed a ratio of B19V DNA concentration in WB divided by B19V DNA concentration in plasma. Samples with positive PCR results that were below the quantitative detection limit of 20 IU/mL B19V DNA were assigned a value of 10 IU/mL.

Donation and/or donor classification

A donor was classified as having chronic persistent infection if two consecutive samples separated by 6 or more months were B19V DNA positive. A donor was classified as recently infected if a donation was IgM antibody positive or if a positive DNA result occurred in a donation given subsequent to a donation that tested B19V DNA, IgM, and IgG negative. A donor was classified as a remote infection if neither of the above criteria applied and if the DNA-positive donation was also IgG positive.

Statistical analysis

B19V DNA concentrations in paired WB and plasma samples were compared using the sign test. The median (and 95% CI) ratio of B19V DNA in WB and plasma was determined. A nonparametric regression of B19V DNA in WB and plasma was performed.

RESULTS

Recovery of B19V DNA from spiked plasma and WB by ultracentrifugation

Quantitative standard curve plasma controls from a previous study were used to validate an ultracentrifugation protocol designed to recover B19 viral particles from plasma and WB preparations. Spiked plasma controls were ultracentrifuged and resulting pellets (which should contain all intact viral particles but not soluble DNA) and supernatant were each tested to evaluate the efficacy of viral DNA recovery. Depending on the dilution assayed, the cycle threshold of the amplification curve derived from the pellet was 6 to 16 cycles earlier than for the supernatant. Since each PCR cycle threshold (C_T) difference corresponds to a twofold difference in starting concentration of the target nucleic acid, these ΔC_T results indicate that the B19 virion particle–associated DNA recovery using the ultracentrifugation protocol was greater than 98% (i.e., only $1/2^6$ to $1/2^{16}$ of B19V DNA remained in the supernatant), thereby establishing the ability to recover virtually all B19 viral particles from plasma samples spiked with B19V standards. Of note, the C_T values observed after amplification of ultracentrifuge pellets derived from each concentration of spiked virus were similar to those of our previous study, in which the spiked plasma samples were directly subjected to the same TC and real-time PCR assay (Table 1 and Kleinmen et al.[16]).

Frozen WB samples spiked with 100,000 IU/mL of B19 virions were then tested to determine the recovery of B19V DNA by the ultracentrifugation protocol. The C_T values of

TABLE 1. Recovery of spiked B19V DNA spiked into plasma using the ultracentrifugation protocol

B19V DNA spike (IU/mL)	Pellet C_T	Supernatant C_T	ΔC_T	% recovery in pellet
1000	30.5	37	6.5	98.9
500	31.5	41	9.5	99.8
250	32.5	40	7.5	99.5
125	33	39	6	98.4
62.5	34	50	16	100

real-time PCR amplifications were compared between the ultracentrifuge pellets and supernatants. In four experiments, the ΔC_T between the pellet (C_T = 22, 26, 24, 20) and supernatants (C_T = 28, 33, 50, 30) were 6, 7, 16, and 10, respectively, indicating B19 viral or DNA recoveries of greater than 98%, similar to the results with comparably spiked and processed plasma controls.

B19V DNA recovery from blood compartments following in vitro spiking of WB

We incubated fresh WB with high-titer B19V–positive plasma from a donor in the acute preseroconversion phase of infection to achieve a final concentration of 100,000 IU/mL WB, thereby simulating a blood specimen acutely infected with B19V. After incubation for 24 hours, WB was processed into three compartments: plasma, RBCs, and WBCs and/or PLTs. The B19V DNA levels were quantified in each of these compartments using sample processing, ultracentrifugation, and TC real-time PCR protocols detailed under Materials and Methods.

The results are presented in Fig. 1. Plasma was used as the reference compartment, and hence data from plasma were plotted as 1.0 in both Fig. 1A and Fig. 1B, with levels of B19V DNA derived from the other compartments plotted as ratios to the corresponding plasma data. The data in Fig. 1A were generated without a wash step and therefore do not include results for "first PBS wash" and "second PBS wash." The concentration of B19V DNA derived from the RBC and WBC and/or PLTs compartments was more than twice the level derived from plasma; in other words, the plasma B19V DNA concentration was less than one-third of the total B19 virus or DNA that had been spiked into WB.

The data in Fig. 1B were generated using a slightly modified protocol incorporating washing of cellular (RBCs and WBC and/or PLTs) preparations before proceeding with lysis of RBCs, pelleting, and B19V DNA extraction. In this protocol, the cells were washed twice with PBS and the amplification results of washing solution specimens were plotted as "first PBS wash" and "second PBS wash." Added together, these two washes yielded about twice the B19V DNA as did plasma. After being washed twice with PBS, very little B19V DNA was recovered in pellets derived from the RBC and WBC and/or PLT compartments. This indicates that after in vitro spiking, approximately two-thirds of B19 virus or DNA is associated with cellular constituents and one-third with plasma, similar to the results derived with the ultracentrifugation protocol using spiked WB preparations, but that this binding is weak and reversible.

Validation of HemoBind and TC protocol

For the in vitro spiking experiments reported above, high concentrations of virus (100,000 IU/mL) were spiked into WB samples to show compartmentalization of the virus. However, this input level is much higher than the concentrations of B19V that we have observed in RADAR donation samples[15,16] and also beyond the limits of our standard curve. When the ultracentrifugation protocol was applied to WB spiked with the standard curve controls, the recovery at 1000 IU/mL was the same for plasma and WB, but the recovery in WB samples decreased and became variable with decreasing viral input compared to the plasma samples (Fig. 2). The WB spiked with the 62.5 IU/mL control yielded poor and erratic recovery of virus (standard deviation ± 4.8 cycles), while the WB spiked with 31.25 IU/mL control yielded no detectable B19V DNA after ultracentrifugation and TC-PCR. Because the ultracentrifugation protocol had low viral recovery at the low end of the WB standard curve, we created a new protocol using HemoBind to process WB samples to enhance the sensitivity of the assay.

Frozen WB standard controls (spiked and nonspiked) were processed using HemoBind to eliminate cellular debris and potential inhibitors of TC and real-time PCR. After HemoBind was added into frozen WB and a clarified solution obtained, the samples were processed by TC and real-time PCR in parallel with plasma standard controls. In Fig. 2, the C_T of real-time PCR amplifications were compared between HemoBind-processed WB samples and the corresponding plasma samples processed using the standard protocol. The slopes for the three procedures (TC and real-time PCR on plasma [TC-RT plasma], the HemoBind and TC real-time PCR on WB [HB-TC-RT WB], and the ultracentrifuged and real-time PCR [UltCfg-RT] on plasma) were –0.99, –0.98, and –1.00, respectively, very close to the theoretical slope of –1.00. The intercepts of TC-RT plasma and HB-TC-RT WB were 39.1 and 39.6, respectively. The similar slopes and intercepts indicate minimal difference between the two protocols, with differential C_T's of only approximately 0.5 cycles.

Comparison of B19V DNA in paired WB and plasma samples from blood donors

Figure 3 summarizes the WB relative to plasma concentrations of B19V for all 104 samples with positive B19V DNA

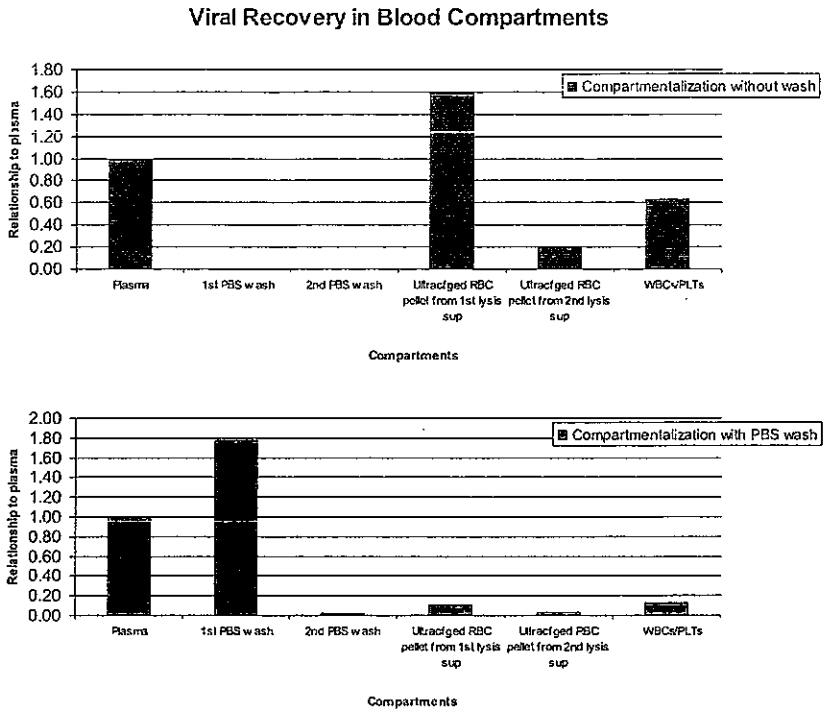


Fig. 1. Distribution of B19V DNA in compartments of WB samples after incubation with B19 virus stock at 100,000 IU/mL. The relative concentrations of recovered B19V DNA are plotted on the *y*-axis using the concentration in the plasma sample as a reference with a designated value of 1.00. The *x*-axis shows the different compartments prepared from the WB samples. Less than one-third of total B19V DNA was present in plasma.

results. Concentrations in WB samples tended to be higher than concentrations in plasma samples ($p = 0.0005$, sign test). For the total sample set, WB samples yielded 1.9-fold higher B19V DNA concentrations than their paired plasma samples (median WB-to-plasma ratio, 1.9; 95% CI, 1.2-4.2). This ratio varied throughout the range of plasma B19V DNA load; for plasma samples with B19V DNA concentrations of greater than 100 IU/mL, this median ratio was 19.5, whereas the ratio was only 1.9 for samples with plasma B19V DNA concentrations of greater than 20 to 100 IU/mL. While the WB-to-plasma ratio for the complete data set is significantly greater than 1, the ratio varies in samples with much higher ratios observed among donor specimens with high plasma B19V DNA levels ($p = 0.01$ by nonparametric regression goodness-of-fit test).

B19V DNA WB-to-plasma ratios at different infection stages

In Fig. 4, B19V DNA WB-to-plasma ratios for 74 blood donations with quantitative (e.g., nonzero) WB and plasma DNA results were analyzed relative to the IgM and IgG antibody status of the donation, which reflects the stage of B19V infection. For 20 IgM-positive, IgG-positive recent infections, the mean ratio was 66.1, with a median of 17.4 and a range of 0.8 to 824.3. For 54 remote infections (IgM negative, IgG positive), this mean ratio was 15.4 with a median of 1.5 and a range of 0.06 to 657.0. The difference in WB-to-plasma B19V DNA ratios between these two stages of infection was significant ($p \leq 0.001$, Mann-Whitney rank-sum test).

Table 2 provides a composite analysis of B19V DNA WB-to-plasma ratios classified by both plasma DNA concentration and IgM status. The highest median ratio (29.7) was found in IgM-positive donations with B19V DNA of greater than 100 IU/mL.

Stage of B19V infection and persistence in blood donors

Figure 5 plots data for the donors with multiple donations from which paired WB and plasma samples were available in the RADAR repository. Figure 5A presents detailed quantitative DNA and antibody results from one representative B19-viremic blood donor with recent infection (IgM positive) that evolved to chronic persistent infection. This donor had repository samples from donations made during both the early and the later stages of B19V infection

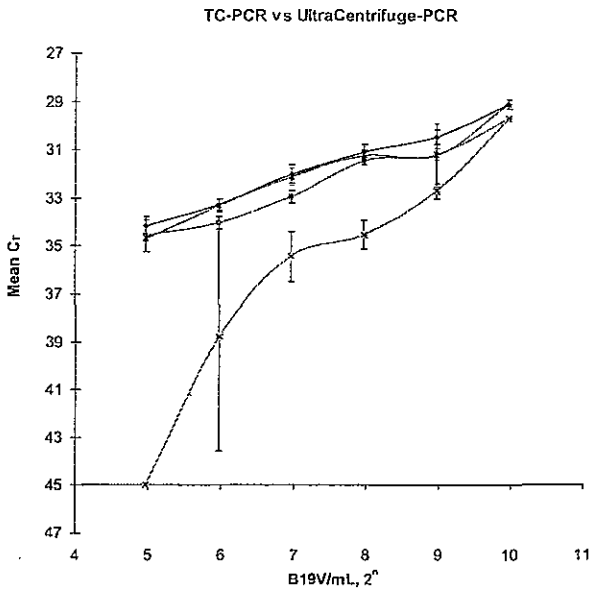


Fig. 2. Viral recovery from ultracentrifugation and HemoBind TC protocols. The *y*-axis shows mean C_Ts (n = 4) with ±1 SD error bars of real-time PCR amplification. The B19 viral input is plotted on the *x*-axis in base 2 log (2^n) format since PCR amplicons theoretically double in quantity per amplification cycle. The control standards are serial twofold dilutions (1000, 500, 250, 125, 62.5, and 31.25 IU/mL). TC-HemoBind WB (■) refers to the protocol using HemoBind, TC, and real-time PCR amplification. TC-Plasma (◆) refers to the protocol using TC and real-time PCR amplification for plasma. UltCfg-Plasma (▲) and UltCfg-WB (⊗) refer to protocols using ultracentrifugation and real-time PCR amplification.

as evidenced by serial donation samples with decreasing IgM reactivity in parallel with increasing IgG seroreactivity. Note that the B19V DNA loads of WB samples at the early stage of infection were significantly higher than those of their paired plasma samples, as indicated by the WB-to-plasma B19V DNA ratios at the top of the figure. In contrast, at the later stages of infection, the WB and plasma B19V DNA levels were almost equal.

Figure 5B shows results for 29 donors whose donations were separated by at least 6 months. Of these, 25 made at least one donation at a greater than 6-month interval from their initial DNA-positive donation. Based on plasma B19V DNA detection, 22 (88%) showed chronic persistent infection; this percentage fell to 72% (18 donors) with the WB assay. Five of the persistently infected donors had additional test results at more than 2 years at which time four donors were B19V DNA negative on both their plasma and their WB samples. All donors with detectable IgM showed loss of the antibody over time. The 14 additional donors (not shown in Fig. 5B), with less than 6 months of follow-up, showed an even higher rate of persistence over the short time frame in which they were evaluated.

Four donors in this study were documented to have incident infection, characterized by a donation that was negative for B19V DNA and antibody followed by a sample that was DNA positive, IgG positive, and in three of four cases, IgM positive (Fig. 5B, and not shown). An additional 12 donors had recent infection based on the detection of IgM antibody in the initial PCR-positive donation (eight cases shown in Fig. 5B; four cases not shown). Three donors with B19V IgG antibody but without detectable B19V DNA on their initial donation showed detectable low-level B19V DNA on their follow-up donation; this may represent either intermittent low-level viremia or a limitation of our assay in consistently detecting such low B19V DNA levels (Fig. 5B, and not shown). Finally, we found one antibody-negative donor with a low level of B19V DNA in both plasma (132 IU/mL) and WB (15 IU/mL); on a subsequent donation given almost 6 months later, this donor tested B19V DNA negative but did not demonstrate antibody seroconversion (case not plotted in Fig. 5B).

DISCUSSION

Because B19V is known to infect and replicate in erythroblasts in the marrow and because mature RBCs contain the B19V receptor (i.e., the P antigen) on their cell surface,[19] we speculated that B19V may be within or bound to circulating erythroid cells and hence viral DNA concentrations might differ in WB and plasma. In addition, previous work with other viral agents had demonstrated that for some viruses that exhibit significant plasma viremia (e.g., human immunodeficiency virus [HIV], hepatitis C virus, West Nile virus, Dengue), a substantial proportion of viral nucleic acid in blood is found to be cell associated when appropriate WB-based nucleic acid test (NAT) is performed.[18-23] Potential mechanisms to explain this finding are the presence of intracellular viral nucleic acid due to active replication in cellular compartments (e.g., HIV DNA and RNA in CD4+T cells) and/or binding of viral particles to cellular constituents of blood, including PLTs (for HIV and Dengue)[19-22] and RBCs (for HIV and WNV).[23,24]

Before evaluating cases of natural B19V infection, we began our study by evaluating different laboratory protocols for B19V WB NAT. WB NAT assays are challenging due to the fact that Hb and other cell-derived constituents inhibit nucleic acid amplification.[25] This applies both to PCR assays (which are inhibited by as little as 1.3 µg/mL of Hb) and to the transcription-mediated amplification assay method commercially developed by Gen-Probe (San Diego, CA). In this latter assay, WB inhibition occurs both at the TC and amplification steps.[26,27] Although a 5- to 10-fold dilution of WB has been demonstrated to overcome this interference, this dilution results in a loss of assay sensitivity.[26,27]

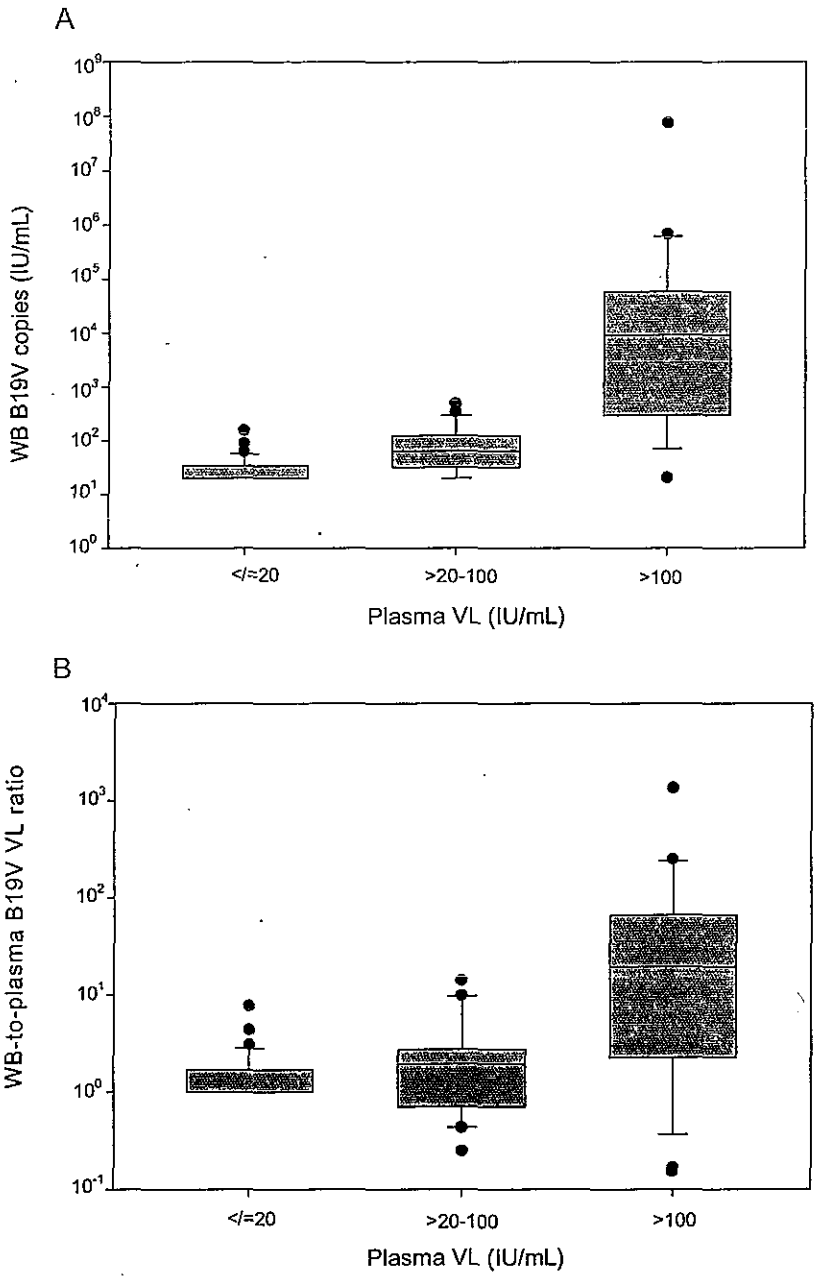


Fig. 3. (A) Box-and-whisker plot of B19V VLs in the WB compartment compared to corresponding plasma VLs. When plasma VL was positive but not more than 20 IU/mL (n = 58), the mean VL in WB was 27.54 IU/mL (median, 20 IU/mL). In the midrange of VL (>20-100 IU/mL, n = 24), the mean WB VL was 100.63 IU/mL (median, 63.2 IU/mL). At the high end, when the plasma VL was greater than 100 IU/mL (n = 22), the mean WB VL was 3.48×10^6 IU/mL (median, 9199 IU/mL). (B) WB-to-plasma VL ratio relative to plasma VL. At the low end of the plasma VL distribution, the WB-to-plasma ratio ranges from 0 to 7.66, with a median of 1 and mean of 1.38. In the midrange (>20-100 IU/mL, n = 24), the WB-to-plasma ratio range was 0.25-13.90, with a median of 1.95 and mean of 2.83. When the plasma VL was greater than 100 IU/mL, the WB-to-plasma ratio range was from 0.15 to 1332, with a median of 19.47 and mean of 103.52.

In this study we evaluated whether ultracentrifugation of blood lysates could quantitatively recover viral particles and overcome these inhibitory effects. We demonstrated that our ultracentrifugation protocol was effective at recovering B19V when high concentrations of B19V were spiked into plasma or WB. Using this protocol, we established that less than one-third of B19V spiked into WB was present in plasma, with the remainder in the cellular compartments and primarily in the RBC compartment.

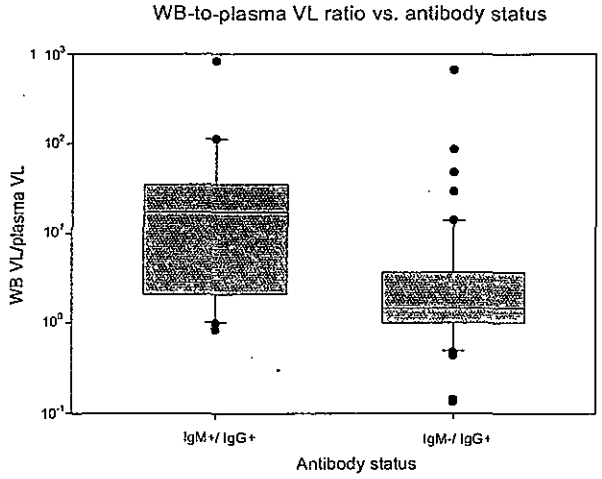


Fig. 4. **Box-and-whisker plot of the B19V DNA ratios of WB samples to plasma samples based on IgM and IgG status, IgM positive and IgG positive (IgM+/IgG+; n = 20) and IgM negative and IgG positive (IgM–/IgG+; n = 54). The B19V DNA ratios are plotted on the *y*-axis and the serologic status is plotted on the *x*-axis. (p > 0.001, Wilcoxon rank-sum test).**

When this cellular compartment was subjected to serial low-speed centrifugation and washing steps, most of the initially bound virus was present in the eluate rather than retained in the cellular preparation. We speculate that the explanation for these results is loose binding of spiked B19V to RBCs, either through weak binding to the P blood group receptor site or through nonspecific binding. These findings are not inconsistent with development of B19 antigen agglutination assays employed in donor screening in Japan, given that those assays employ optimized conditions to maximize binding and they require more than 10^{10} B19 particles/mL of plasma for positive results.[28,29]

The above spiking and recovery studies were performed using a high concentration of B19V. Further experiments demonstrated that the ultracentrifugation protocol was not efficient in recovering B19V when lower concentrations of virion particles were spiked into WB. We speculate that the difference in the protocol efficiency at high and low viral spiking concentrations was due to the need to have sufficient concentrations of virus to create ultracentrifugation pellets that could withstand subsequent washing and recovery procedures. Use of unrelated carrier virus particles or microbeads to facilitate B19V recovery at low spiking levels could potentially overcome the sensitivity problem of the ultracentrifugation protocol (as discussed in Lee et al.[20]).

As part of our ongoing NAT research, we developed a new WB processing protocol using a novel buffer to precipitate and eliminate the Hb and other proteins which inhibit TC and PCR or transcription-mediated amplification. This unique buffer and associated protocol is termed "HemoBind" (patent pending) and delivers viral nucleic acids in a supernatant in a sufficiently pure form so that

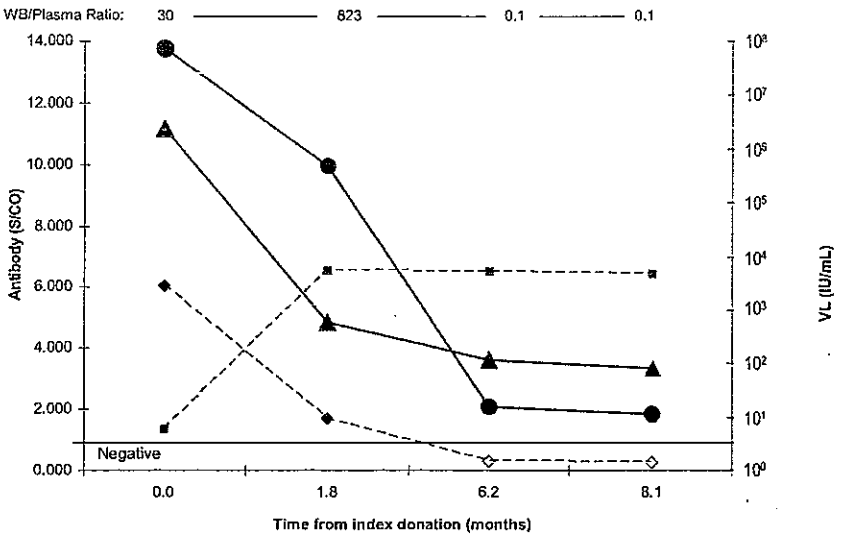
TABLE 2. WB-to-plasma ratios for units with quantitative B19V DNA results of greater than 20 IU/mL

	WB-to-plasma DNA ratio					
	IgM positive			IgM negative		
Plasma DNA concentration	Number	Mean	Median	Number	Mean	Median
>20-100	6	4.43	2.15	18	2.19	1.06
>100	14	137.3	29.7	8	44.4	2.64
Total*	20	97.4	13.9	26	15.2	1.82

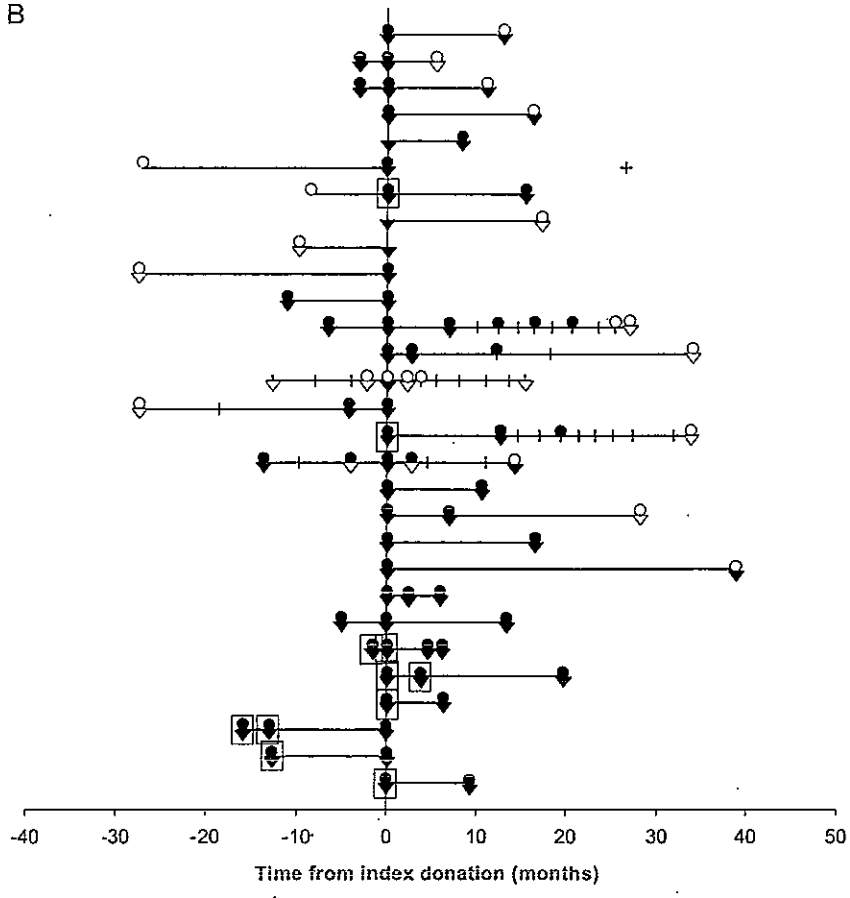
* Data for 58 IgM donations with DNA results that were negative or less than 20 IU/mL are not included.

Fig. 5. **B19V distributions in blood compartments and persistence in infected RADAR donors. (A) B19V DNA concentration and serologic status over time for a blood donor with chronic persistent B19V infection. IgM (◆) and IgG (■) signal-to-cutoff (S/CO) ratio is plotted on the left *y*-axis, while the corresponding plasma (▲) and WB (●) B19V DNA concentrations in IU/mL are plotted on the right *y*-axis. The timing of the donation events is plotted on the *x*-axis. The WB-to-plasma ratio is indicated above the corresponding donation. (B) Quantitative B19V DNA results for all tested donations from 29 donors with at least one donation at a greater than 6-month interval. The *x*-axis represents time from index donation with the index donation plotted as Time 0. Additional donations going backward or forward from a positive index donation were tested by quantitative PCR, performed on both WB (●, ○) and plasma samples (▼, ▽). The closed symbols denote samples with detectable B19V DNA results and the open symbols denote negative B19V DNA results. IgM-positive donations are indicated by boxed symbols.**

A


WB/Plasma Ratio: 30 823 0.1 0.1
14.000
12.000
10.000
8.000
6.000
4.000
2.000
0.000
Antibody (S/CO)
Negative
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
VL (IU/mL)
0.0
1.8
6.2
8.1
Time from index donation (months)

B


-40
-30
-20
-10
0
10
20
30
40
50
Time from index donation (months)

there is no inhibition of either the TC procedure or the subsequent amplification steps. Through spiking experiments, we demonstrated that combining HemoBind with TC and real-time PCR amplification yielded a quantitative B19V DNA assay that was efficient and reproducible in recovering B19V DNA spiked into WB over a wide spectrum of spiking concentrations.

We applied the HemoBind TC real-time PCR assay to paired WB and plasma samples from serial donations of RADAR donors who had been shown to have detectable plasma B19V DNA. During early stages of B19V infection when IgM was present and when B19V DNA concentrations were higher, we demonstrated that the median WB B19V DNA concentration was approximately 30 times higher than in plasma, and in some samples the WB-to-plasma ratio was more than 1000. In contrast, when IgM was absent later in infection and when B19V DNA concentrations were lower, the median WB-to-plasma ratio varied only by a factor of 1.06. Possible explanations for these results include the following: B19V is preferentially bound to RBCs when it is present in IgM immune complexes, more B19V is bound at higher plasma concentration due to steric effects on receptor-mediated binding, or B19V DNA is present at high levels within a subset of peripheral blood RBCs in the earlier compared to the later stages of B19V infection. This last hypothesis is intriguing given that B19V propagates in erythroblasts and the period of high-level cell-associated B19 viremia corresponds roughly to the 120-day survival period of RBCs in peripheral blood, and hence RBCs derived from infected erythroblasts could circulate for several months harboring B19V and account for the differential partitioning of DNA during the convalescent phase of infection. Further experiments that will be needed to investigate these possible explanations would require access to fresh blood specimens from acutely infected subjects to allow purification of intact cell subsets by centrifugation and elutriation or flow cytometry–based sorting, coupled with quantitative B19V detection analyses.

Although B19V infection had been classically considered to result in an acute transient infection, recent studies have established that chronic asymptomatic as well as symptomatic persistent B19V infections occur in a proportion of infected persons.[2-7] Such infections are characterized by prolonged periods with low levels of B19V DNA in blood of IgG-seropositive donors and patients. The rate of occurrence and determinants of persistent B19V infections are poorly understood. A few patients have been reported to have asymptomatic persistent B19 viremia for up to 3 to 5 years using highly sensitive PCR assays. Prolonged persistence of low-level plasma viremia has also been observed in follow-up studies of healthy donors.[2-7] In addition, recent studies indicate that B19V DNA can persist in solid tissues for years or even decades after clearance of circulating viremia and seroconversion.[5]

In this study, using a highly sensitive B19V DNA assay, we demonstrated that persistent B19V infection occurred in 88% of evaluable blood donors who had given at least one sample more than 6 months after plasma B19V DNA was initially detectable. B19V DNA levels either decreased from higher levels or remained at consistently low levels in these donors. These findings are similar to those in blood donor studies reported recently from Germany and Japan.[6,7] In addition, we found that B19V DNA persistence could also be detected by our WB assay, but without incremental detection relative to plasma; this is explained by the low titers of virus and absence of IgM during the persistent stage of infection.

While almost all donors demonstrated findings consistent with the known natural history of B19V infection, we did identify one donor with unusual results. B19V DNA was detectable at low levels in the absence of IgM and IgG antibody, which is contrary to the usual high viral loads (VLs; $>10^6$ IU/mL) that are detectable prior to antibody seroconversion. In addition, this donor did not seroconvert to IgG on a follow-up sample as would be expected. It is possible that this donor had a transient B19V infection (as indicated by the low DNA concentration) but for unknown reasons did not develop antibody to the VP2 antigen that is detectable by the antibody kits used in this study. We do not think the results can be explained by laboratory error since the positive index donation DNA results were obtained on both plasma and WB samples from this donation.

Since B19V is only rarely transmitted by blood transfusion, our finding of differential (high) WB-to-plasma concentrations in the early IgM-positive stages of infection have limited if any significance for blood safety. However, we believe that the general issue of differential levels of WB versus plasma nucleic acid concentrations for viruses and other blood-borne pathogens is important and encourage further development of sample preparative methods to enable performance of NAT on WB specimens. For transfusion-transmissible viruses with infectious window periods not currently detected by NAT screening, it is possible (though speculative) that WB nucleic acid testing could achieve greater sensitivity than plasma testing and thereby offer a tool to decrease residual risk. WB methods could also enable detection of persistent viral nucleic acids in convalescent or low-level persistent stages of infection (e.g., "occult HBV" and "elite controller HIV" infections), similar to our findings of more efficient detection of B19V during the convalescent IgM-positive phase of infection and the reported more efficient detection of WNV in WB relative to plasma in asymptomatic donors.[24] Also, adoption of high-throughput sample processing to allow use of WB in NAT assay systems would open the door to detection of cell-associated viruses and of parasitic and other nonviral agents, including those that are internal to RBCs (e.g., malaria or babesia para-

sites) or that partition with WBC fractions (e.g., *Trypanosoma cruzi*).

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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識別番号・報告回数			報告日	第一報入手日 2011 年 10 月 13 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	Vox Sanguinis 2011; Article published on line 22 JUL	公表国 日本	
販売名 (企業名)	①テタノブリン IH 静注 250 単位　(ベネシス) ②テタノブリン IH 静注 1500 単位　(ベネシス) ③テタノブリン筋注用 250 単位　(ベネシス)					

研究報告の概要 -71-

背景と目的：
我々は以前にパルボウイルス B19 遺伝子型 1 は血漿製剤由来の異なる溶液の液状加熱において異なる熱感受性パターンを示したことを報告した。本研究では、同様に B19 遺伝子型 2 を調べた。

材料と方法：
B19 の遺伝子型 1 と遺伝子型 2 の DNA を含む 2 つの血漿検体を用いた。アルブミン、免疫グロブリン、ハプトグロビンとアンチトロンビンの製造における熱処理工程の直前に採取した 4 つの工程検体に B19 をスパイクし、その後 60℃-10 時間の加熱処理をした。低 pH 免疫グロブリン溶液も B19 をスパイクし、室温で 14 日間の処理をし、感染性を測定した。

結　　果：
B19 遺伝子型 1 同様に、遺伝子型 2 は不活化の 3 つのパターンを示した。
(i)アルブミンと免疫グロブリンの急速な不活性化
(ii)ハプトグロビンの遅い不活性化
(iii)アンチトロンビンのみに限定した不活性化
低 pH 免疫グロブリン溶液中での感受性もまた、遺伝子型 1 に類似していた。

結　　論：
B19 の遺伝子型 1 と 2 の両方は、異なる血漿製剤の間で液状加熱と低 pH に対する感受性が変化した。

使用上の注意記載状況・その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
1. 慎重投与
(1)略
(2)略
(3)溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(4)免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
6. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎

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報告企業の意見	今後の対応	児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕
万一、本剤の原料血漿にB19が混入したとしても、CPVをモデルウイルスとしたウイルスバリデーション試験成績及びB19を用いた不活化･除去試験の結果から、製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。	

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ORIGINAL PAPER

Variability of parvovirus B19 genotype 2 in plasma products with different compositions in the inactivation sensitivity by liquid-heating

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Vox Sanguinis

Background and Objectives Our previous report showed that parvovirus B19 genotype 1 in different solutions derived from plasma preparations showed different heat-sensitivity patterns during liquid-heating. In this study, we similarly examined B19 genotype 2.

Materials and Methods Two plasma samples one containing B19 genotype 1 and the other genotype 2 DNA were used. Four process samples collected immediately before the heat treatment step in the manufacture of albumin, immunoglobulin, haptoglobin and antithrombin preparations were spiked with B19 and subsequently treated at 60°C for 10 h. A low pH immunoglobulin solution was also spiked with B19 and treated at room temperature for 14 days. Infectivity was then measured.

Results B19 genotype 2, similar to genotype 1, showed three patterns of inactivation: (i) a rapid inactivation in the albumin and immunoglobulin preparations, (ii) a slow inactivation in the haptoglobin preparation and (iii) only limited inactivation in the antithrombin preparation. Its sensitivity in the low pH immunoglobulin solutions also resembled that of genotype 1.

Conclusion Both genotypes 1 and 2 of B19 varied in sensitivity to liquid-heating and low pH among different plasma preparations.

Key words: genotype 2, heat & pH sensitivity, parvovirus B19, plasma products, viral inactivation.

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Introduction

Human parvovirus B19 (B19) is not highly pathogenic to healthy individuals but can have serious effects in pregnant women and immunosuppressed individuals.

B19 has been long considered the only human pathogen in the genus *Erythrovirus* in the family *Parvoviridae* [1]. Its DNA sequence had been regarded as extremely stable, with variation of only approximately 1–2%. However, new variants (V9, A6 and Lali) have been reported to differ from genotype 1 by approximately 10% [2–4]. Genotypes 2 and 3 seem to have similar virulence to genotype 1 [5]. Servant *et al.* [6] proposed classifying human erythroviruses into genotypes 1 (prototype Au), 2 (Lali-like) and 3 (V9-like). Genotype 1, the major form of B19, has spread worldwide. Genotype 2 has been reported mostly in Europe and North America. At present, only small numbers of donor plasma samples are believed to contain high levels of genotype 2 [7]. In genotype 1, the structural proteins of the capsid consist of approximately 96% VP2 (58 kDa) and 4% VP1 (84 kDa). VP1 differs from VP2 only in a unique N-terminal region (VP1u), where it has an additional 227 amino acids

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[8]. However, there have been no reports on morphological structure and protein composition in genotype 2.

B19 genotype 1 DNA has been detected by PCR in plasma products, such as coagulation factors [9, 10]. Genotype 2 DNA has also been detected in 2·5% of coagulation factor concentrates [11]. Despite measures taken against viral contamination, including improved testing and inactivation and/or removal steps during the manufacturing process, the potential for B19 transmission through plasma products remains. B19 genotype 1 has been shown to be sensitive to liquid-heating than other animal parvoviruses such as canine parvovirus (CPV) and porcine parvovirus (PPV) [12–15]. We have also shown that B19 genotype 1 in different solutions showed different heat-sensitivity patterns during liquid-heating: (i) a rapid inactivation in albumin or immunoglobulin preparations, (ii) a slow inactivation in haptoglobin preparations and (iii) only limited inactivation in antithrombin preparations [14]. Genotype 2 was also shown to be rapidly inactivated in 5% albumin at 56°C [16]. The effective inactivation of both genotypes in coagulation factors, which contained 7–8% residual moisture, during vapour-heating (STIM-4) at 60°C for 500 min followed by at 80°C for 60 min has also been reported [17]. In the case of freeze-dried products, the sensitivity of B19 genotype 1 to heat treatment has been shown to be influenced by the residual moisture in the sample [18]. Therefore, B19 genotype 2 seems to be effectively inactivated under high residual moisture and high temperature conditions. However, it remains unknown whether the heat sensitivity of genotype 2 varies in different types of products.

In this study, we examined the inactivation kinetics of genotype 2 during liquid-heating and low pH treatment in several manufacturing processes using a recent B19 isolate derived from rejected donor plasma containing B19 genotype 2. In addition, the full nucleic acid sequence, the morphological structure, and the protein composition of the genotype 2 isolate also investigated.

Materials and methods

Viruses

Two plasma samples containing either B19 genotype 1 or genotype 2 DNA, collected from donors in the USA and named F15 and F27, respectively, were used. Each sample was converted to serum by the addition of calcium chloride. The amount of genome in F15 and F27 was quantified as 11·2 and 11·0 Log_{10} IU/ml, respectively (Artus™ Parvo B19 TM PCR kit; Artus GmbH, Hamburg, Germany). The samples were also examined for viral markers as described below, according to good manufacturing practices. They tested negative for anti-human immunodeficiency virus (HIV)-1/2 IgG, hepatitis B virus (HBV) surface antigen, anti-hepatitis C virus (HCV) IgG and anti-B19 IgG in immunoassays. In addition, HIV RNA, hepatitis A virus RNA, HBV DNA and HCV RNA were not detected using nucleic acid amplification tests.

Test materials

In this study, we used a 25% albumin preparation containing sodium caprylic acid/acetyl tryptophan, a haptoglobin preparation containing glycine, an antithrombin preparation containing sodium chloride/tri-sodium citrate dihydrate and an IVIG preparation containing D-sorbitol, respectively, as stabilizers. To mimic the manufacturing process conditions, test samples were collected immediately before the heat treatment of the albumin, haptoglobin and antithrombin preparations (Albumin25%$_{I.V.-BENESIS}$, Haptoglobin$_{I.V.}$2000$_{units-BENESIS}$ and Neuart®$_{I.V.}$, respectively; Benesis Corporation, Osaka, Japan). To avoid interference with the infectivity assay by neutralizing antibody against B19 during heat or low pH treatments, an immunoglobulin sample derived from anti-B19 IgG-negative plasma was prepared under the same manufacturing conditions as Venoglobulin®IH5%$_{I.V.}$ (Benesis Corporation). The samples were collected immediately before the heat or low pH treatment.

For neutralization assays, a commercial product, intravenous immunoglobulin (IVIG, Venoglobulin®IH5%$_{I.V.}$ including anti-B19 IgG), was used.

Inactivation and neutralization of viruses

The process samples described above were investigated by liquid-heating as described previously [13–15]. The samples spiked with B19 were divided into aliquots and heat treated in water bath at 60°C for preset periods. The collected samples were immediately cooled to make a 10-fold serial dilution and incubated with the KU812 cells. The infectivity remaining in each sample was measured by the subsequent detection of spliced B19 mRNA in cultured cells, as described [13, 14]. Two independent inactivation experiments were performed. The viral titre (log_{10}) in one experiment was determined by non-detectable end-point dilution [13] and in the other experiment by tissue culture infectivity dose 50% ($TCID_{50}$) according to the Kärber method [14], respectively.

The forward primers and the reverse primers were defined as follows. The primer set of B19-25 (nt 1897–1916; 5′-GTCGGAAGCCCAGTTTCCTC-3′ as 5′ sense) and B19-11 (nt 2962–2943; 5′-TGCACCAGTGCTGGCTTCTG-3′ as 3′ antisense) was used for the non-detectable end-point dilution method, and another primer set of B19-21 (nt 1961–1980; 5′-TGGCAGACCAGTTTCGTGAA-3′ as 5′ sense)

and B19-22 (nt 2886–2866; 5′-CCGGCAAACTTCCTTGAAAA-3′ as 3′ antisense) was used for $TCID_{50}$.

Both viruses were also tested for inactivation at low pH. The viruses were incubated in IVIG sample (pH 4·15), IVIG buffer without IgG (5% Sorbitol, pH 4·15) or cell culture medium at room temperature for a preset period. The low pH reaction was stopped by addition of 1:9 volumes of culture medium. Run control studies comprising the same experimental conditions without the virus inactivation step were also performed in each study as a control. Cytotoxicity tests were also performed, and infectivity was then measured in the non-cytotoxic range as described above.

Neutralization titres of IVIG samples against B19 viruses were measured as previously reported [15].

Cloning and sequencing of the B19 genotype 2 genome

Restriction enzymes, the DNA polymerase I Klenow fragment and a DNA Ligation kit, Ver. 2 (Takara Bio Inc., Shiga, Japan), were used for cloning. Viral DNA was extracted from F27 without amplification using Smitest Ex-R&D (GenomeScience Laboratories Co., Fukushima, Japan). The DNA was cleaved by cutting inverted terminal repeats (ITRs) in half with *BssH*II, treated with the Klenow fragment to fill the 3′-end, and ligated into the *Hinc*II site of pUC19. The ligated DNA was transfected into competent DH5α cells (Toyobo Co. Ltd., Osaka, Japan). Each clone was sequenced using a 3130*xl* genetic analyzer (Applied Biosystems Japan Ltd, Tokyo, Japan) and BigDye Terminator Cycle Sequencing kit (Applied Biosystems Japan Ltd). Full-length sequences were obtained by primer walking. The entire sequence of F27 has been deposited in the DDBJ/GenBank/EMBL nucleotide databases (accession number AB550331).

Electron microscopy and sodium dodecyl sulphate (SDS)–polyacrylamide gel electrophoresis (PAGE)

Each plasma sample was diluted threefold with phosphate-buffered saline (PBS) and then ultracentrifuged at 154 000 *g* for 3 h. The precipitate was resuspended with PBS, and the viral fraction in the suspension was purified by caesium chloride density gradient ultracentrifugation at 210 000 *g* for 26 h. The fractions were collected, and the amount of B19 DNA in each was quantified using an Artus™ Parvo B19 TM PCR kit (Artus GmbH).

For transmission electron microscopy (TEM), the B19 DNA-rich fractions were dialysed in 50 μM ammonium acetate overnight and 8% paraformaldehyde in 50 μM ammonium acetate added to a final concentration of 2% to inactivate the virus. Specimens for negative staining were made as follows; inactivated samples were applied to carbon-coated electron microscope grids (200-A copper grid; Nisshin EM Co., Tokyo, Japan) for 5 min, and then the excess was removed by blotting with filter paper. Once on the carbon-coated grids, samples were stained with 2% (w/v) uranyl acetate for 30 s, blotting with filter paper and dried in air. Negatively stained specimens were observed by TEM at 100 kV (JEM-100S; JEOL Datum Ltd., Tokyo, Japan). Whole micrographs were digitized as 8-bit images using a scanner (GF ×800; Seiko EPSON Co., Nagano, Japan) and then analysed with the software EMAN (version 1.8, http://blake.bcm.tmc.edu/eman/eman1/) and ImageJ (version 1.36b, http://rsb.info.nih.gov/ij/index.html).

The B19 DNA-rich fractions were precipitated with 10% (w/v) trichloroacetic acid, and the precipitates were solubilized in a buffer containing 2% SDS, 5% (v/v) 2-mercaptoethanol, 0·01% (w/v) bromophenol blue and 20% (w/v) glycerol for 3 min at 100°C. Aliquots were electrophoresed in 10% polyacrylamide gel (Bio-Rad Laboratories, K. K., Tokyo, Japan) and subjected to Coomassie brilliant blue (CBB) staining.

Results

Inactivation of B19 genotypes 1 and 2 during liquid-heating

The infectivity of B19 genotype 2 (F27 isolate) in each sample was measured by detecting B19 spliced mRNA in infected KU812 cells. The B19 genotype 2 virus was rapidly inactivated at 60°C in the 25% albumin and immunoglobulin preparations by at least 4·6 logs (Fig. 1a, b). It was slowly inactivated in the haptoglobin preparation (Fig. 1c). The virus was only partially inactivated, i.e. 2·6 logs in the antithrombin preparation even after 10 h at 60°C (Fig. 1d). The viruses showed retained infectivity in the run control study (data not shown). Interestingly, the inactivation kinetics of genotypes 1 and 2 in each product were almost the same.

Inactivation of B19 genotypes 1 and 2 at low pH

The B19 virus samples were also incubated in the immunoglobulin preparation (pH 4·15). Both genotype 1 and 2 viruses were inactivated below detectable limits, i.e. by >3 logs at day 1 (Fig. 2). Both B19 viruses in the IVIG sorbitol buffer, used as a control, were also inactivated beyond detection at day 7. B19 infectivity was retained in the run control study (data not shown). The kinetics demonstrated that genotypes 1 and 2 showed similar sensitivity to low pH treatment.

Neutralizing activity of IVIG against genotype 2

IVIG showed neutralizing activity against genotype 2 as well as genotype 1 (Table 1). The result suggested that

anti-B19 genotypes 1 and 2 neutralizing IgG was contained in the IVIG products.

Cloning and sequencing of the genome of isolate F27

The viral genome of F27 was cloned. The nucleotide and amino acid sequences were compared with those of strains Au (genotype 1; GenBank accession No. M13178), A6 (genotype 2; GenBank accession No. AY064475) and V9 (genotype 3; GenBank accession No. AX003421). The homology between F27 and the Au, A6 and V9 strains was 87·7%, 98·1% and 91·1%, respectively. The amino acid sequences of VP1 and VP2 were highly conserved (97·4% and 98·6%) between F27 and Au.

It has been reported that four basic regions in VP1u exist among animal parvoviruses [CPV, PPV and minute virus of mice (MVM)] [19]. The overall percentage of basic amino

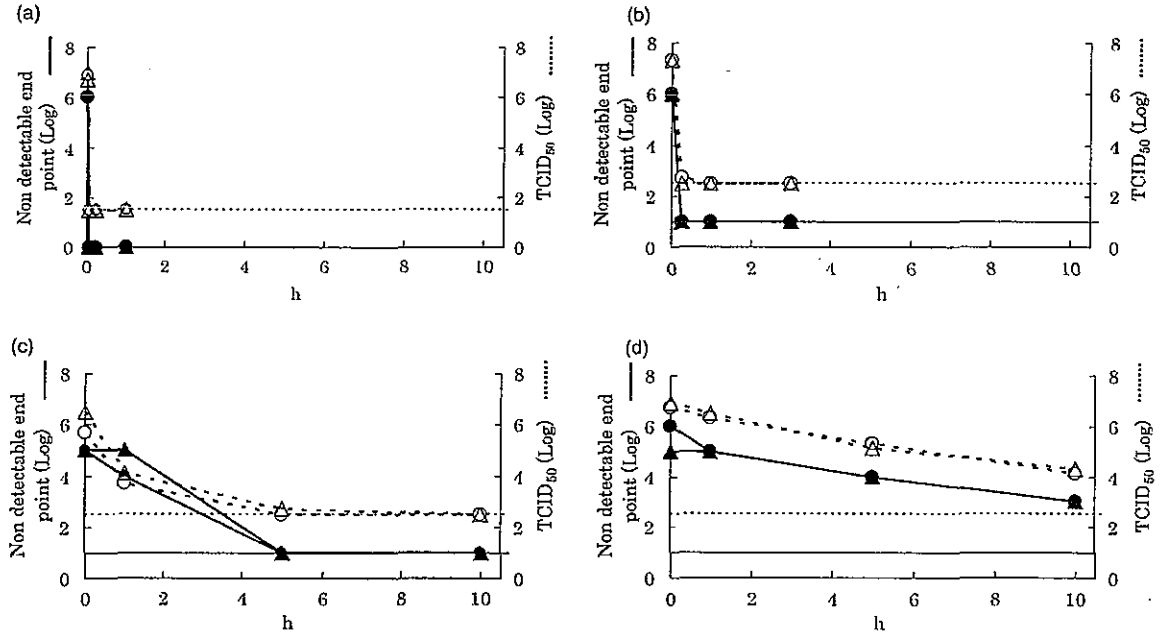

Fig. 1 The kinetics of heat-induced inactivation of B19 genotypes during the manufacturing process. Samples spiked with B19 were collected immediately before and after heat treatment at 60°C using 25% albumin (a), immunoglobulin prepared from anti-B19 immunoglobulin-negative plasma (b), haptoglobin (c) and antithrombin (d) preparations. The infectivity remaining in each sample of B19 was measured by non-detectable end-point dilution [genotype 1 (●) and genotype 2 (▲), solid lines] and by $TCID_{50}$ [genotype 1 (○) and genotype 2 (△), dashed lines], respectively, as described in Materials and Methods. The solid and dotted horizontal lines represent the detection limits of the assays.

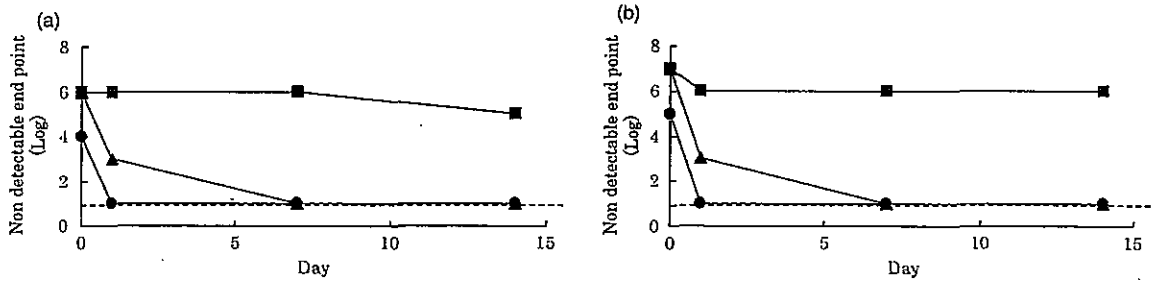

Fig. 2 The kinetics of low pH-induced inactivation of B19 genotype 1 strain F15 (a) and genotype 2 strain F27 (b) during the IVIG manufacturing process. The infectivity remaining in each sample of the immunoglobulin preparation (pH 4·15) derived from anti-B19 IgG-negative plasma (●), 5% sorbitol buffer control (pH4·15; ▲) and culture medium pH7 (■) was measured by non-detectable end-point dilution with two independent experiments. The dashed horizontal lines represent the detection limit.

Table 1 Neutralization of B19 genotypes with intravenous immunoglobulin

Dilution (fold)	Total IgG/ reaction (μg)	Total antibody titre (IU)	Genotype 1 DNA (9·7 log copies)	Genotype 2 DNA (9·7 log copies)
1	2500	13·90	–	–
3	830	4·63	–	–
9	280	1·54	–	+
27	90	0·51	+	+
81	30	0·17	+	+

+, Infectious to KU812 cells; –, non-infectious to KU812 cells.
Total reaction volume was 0·6 ml.

acids (arginine and lysine) in the viruses including F27 is summarized in Table 2. Interestingly, 8·8% of basic amino acids with no basic region in VP1u of F27 and 8·8% of basic amino acids with only one basic region in Au were also confirmed.

Electron microscopic observation and protein composition of viral particles

Native particles of genotype 2 were first observed by TEM (Fig. 3). The genotype 1 and 2 particles were of similar shape: mostly hexagonal and sometimes pentagonal. Mean diameters were measured at the shorter axis and were 22·0 ± 1·9 nm (n = 155) and 22·5 ± 1·5 nm (n = 242), respectively.

Native particles were also subjected to SDS–PAGE in a 10% gel, followed by staining with CBB (Fig. 4). Both genotype 1 and 2 particles also comprised the major 58-kDa protein VP2 and minor 84-kDa protein VP1. Western blotting using the anti-B19 polyclonal antibody confirmed that these proteins are viral structural components (data not shown).

Table 2 The numbers of basic amino acids and basic regions within the VP region of three animal parvoviruses and parovirus B19 genotypes 1 and 2

	Basic amino acids/total amino acids		Number of basic regions	
	VP1u	VP2	VP1u	VP2
CPV	29/167 (17·4)	43/584 (7·4)	4	2
PPV	29/170 (17·1)	38/579 (6·6)	4	0
MVM	24/142 (16·9)	44/587 (7·5)	4	1
B19 genotype 1	20/227 (8·8)	42/554 (7·6)	1	0
B19 genotype 2	20/227 (8·8)	42/554 (7·6)	0	0

The GenBank accession numbers of the five isolates are M19296 (CPV; strain CPV-N), D00623 (PPV; strain NADL-2), J02275 (MVM; strain MVM(p)), M13178 (B19 genotype 1 strain Au) and AB550331 (B19 genotype 2 isolate F27).
The values in parentheses are shown as percentages.
CPV, canine parvovirus; MVM, minute virus of mice; PPV, porcine parvovirus.

Discussion

In this study, we have shown that the heat-sensitivity patterns of B19 genotype 2 varied in different solutions during liquid-heating: (i) rapid inactivation in albumin and immunoglobulin preparations, (ii) slow inactivation in haptoglobin preparations and (iii) only limited inactivation in antithrombin preparations. The results revealed that in terms of sensitivity to heat, B19 genotype 1 and 2 viruses are similar to each other. The stabilizers in plasma preparations are selected based on the ability to stabilize plasma proteins. The composition of the test sample is an important factor in the evaluation. Thus, to mimic manufacturing conditions, samples were collected just prior to the heating process.

Only limited B19 inactivation occurs in some products during liquid-heating. Therefore, to further improve the safety of plasma products, different or additional methods of inactivation and/or removal are desirable [20]. In our albumin, IVIG, haptoglobin and antithrombin preparations, robustness was ensured by employing steps other than heating. A 15-nm filter is used for the antithrombin preparation. Observations by TEM showed the mean diameter of B19 genotype 1 and 2 particles to be 22·0 and 22·5 nm, respectively. Previous studies have shown that filtration of a haemoglobin solution resulted in a more than 6-log reduction in B19 genotype 1 [21]. Given the similarity in size, the 15-nm filter would be effective for the removal of genotype 2 as well as genotype 1 particles. B19 genotype 1 also showed heat resistance properties in sodium chloride/tri-sodium citrate dehydrate buffer whereas the virus was inactivated rapidly in sodium chloride or tri-sodium citrate dehydrate buffer alone (data not shown). The results suggested that the heat sensitivity of the B19 is affected by a number of factors and appears to be complex. Thus, B19 heat sensitivity during heat treatment should be evaluated for in each process condition to avoid an over estimate of the level of B19 inactivation.

Recently, information on the mechanisms by which viruses are inactivated during liquid-heating has been obtained using B19 and MVM. The genomes of B19 and MVM were released with increasing temperature and subsequently only empty capsids remained. Interestingly, B19 was released at a lower temperature than MVM. This phenomenon seemed to be related to the heat inactivation kinetics of B19 and MVM [22, 23]. In addition, the VP1u region of B19 or MVM seemed to remain inaccessible in the capsid before heat treatment, because an anti-B19 VP1u antibody could not bind to viral particles, whereas it could

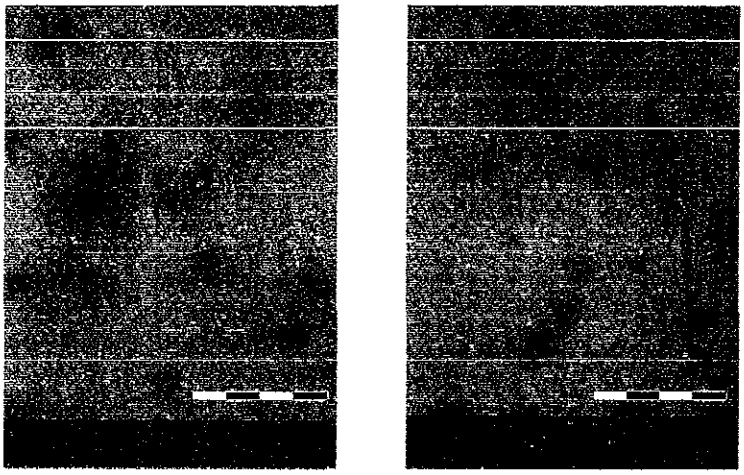

Fig. 3 Electron micrographs of purified native B19 particles. Field view of genotype 1 strain F15 (left) and genotype 2 strain F27 (right). The bar represents 400 nm. Inlet shows isolated particles. Each rectangle is 108 nm wide and 87 nm high.

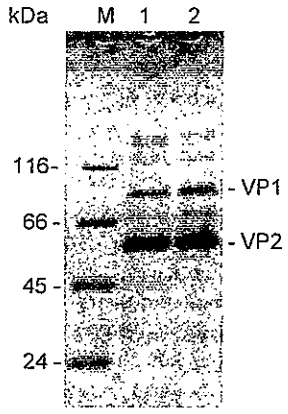

Fig. 4 Coomassie brilliant blue staining of the structural proteins of native particles of B19 genotypes 1 and 2. Purified viral particles were reduced and subjected to SDS-PAGE. Lanes M, 1 and 2 show the molecular marker, genotype 1 strain F15 and genotype 2 strain F27, respectively.

after heating. It was suggested that VP1u became exposed on the surface of particles during heating [24, 25]. VP1u may be involved in movement through the pores of the nuclear membrane in animal parvoviruses, because it is rich in basic amino acids (arginine and lysine), which is typical of many DNA-binding proteins [19]. The percentage of basic amino acids in the major capsid protein VP2 of CPV, PPV and MVM was 6·6–7·5%. That in VP1u was around 17%.

Although absolute molecular sizes were not clarified, we confirmed that VP1 and VP2 of B19 genotype 2 were similar in size to genotype 1 by SDS-PAGE of viral particles. Interestingly, the percentages of basic amino acids in VP1u between B19 genotypes and animal parvoviruses were significantly different. The basic region in VP1u and binding with viral DNA in animal parvoviruses may be involved in the stabilization during heat inactivation. Therefore, the different properties of the binding of VP1u with viral DNA may influence the heat sensitivity of B19.

The inactivation kinetics of genotype 2 at low pH was almost the same as that of genotype 1. Blümel *et al.* [16] also reported similar properties. The inactivation mechanism at low pH was also considered, to be similar to that for heat treatment [21–23].

The neutralizing activity of IVIG against genotypes 1 and 2 was similar. This is because of the presence of a wide range of neutralizing antibodies in IVIG derived from over 10 000 healthy donors [26]. When a renal transplant recipient infected with B19 genotype 2 was treated with high-dose IVIG, the viral load in serum was reduced and the symptoms improved [27]. This clinical study also suggests that IVIG contains neutralizing activity towards the genotype 2 virus.

In conclusion, the inactivation kinetics on liquid-heating depends on the composition of the solution, and low pH treatment of native B19 genotype 2 virus had a remarkably similar effect to that on genotype 1. Although B19 is classified in the family *parvoviridae*, it differs from other animal parvoviruses in that it is more variable to inactivation. Further study is needed to clarify the mechanism behind the heat resistance of B19.

Acknowledgements

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識別番号・報告回数			報告日	第一報入手日 2011 年 10 月 13 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②ポリエチレングリコール処理抗破傷風人免疫グロブリン ③乾燥抗破傷風人免疫グロブリン	研究報告の公表状況	Journal of Virological Methods 2011;178(1-2):39-43	公表国 日本		
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-80- 研究報告の概要

製造工程の異なる段階での生物学的製剤の検査は、現在では定量ポリメラーゼ連鎖反応(Q-PCR)ベースの分析を含む。Q-PCR 技術は、ウイルス粒子内のウイルスゲノムだけでなく、検体中の分解したゲノムの断片をも検出可能である。15～19nm フィルターのウイルス除去能について、最小のノン・エンベロープウイルスの一つであるパルボウイルス B19(B19)を用いて、感染力分析と Q-PCR 分析を行うことにより調査した。フィルター処理された検体は感染力を示さなかったが、ウイルス DNA は Q-PCR によって検出された。
興味深いことに、15nm 濾液中の全ウイルスゲノムの約 90%は、Q-PCR により 0.5kb 未満の検出可能サイズを有していた、結果としてリダクションファクターは Q-PCR を用いて過小評価されていた。
Q-PCR を用いたリダクションファクターは、遊離 B19 DNA の大量の存在により過小評価されるかもしれない。
従って、Q-PCR の結果は慎重に解釈されるべきである。Q-PCR によるウイルス DNA の断片から増幅を排除するためには、プライマーの慎重な設計が必要である。

使用上の注意記載状況・
その他参考事項等

代表としてテタノブリン IH 静注 250 単位の記載を示す。
1. 慎重投与
(1)略
(2)略
(3)溶血性・失血性貧血の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、発熱と急激な貧血を伴う重篤な全身症状を起こすことがある。〕
(4)免疫不全患者・免疫抑制状態の患者〔ヒトパルボウイルス B19 の感染を起こす可能性を否定できない。感染した場合には、持続性の貧血を起こすことがある。〕
2. 重要な基本的注意
(1)略
1)血漿分画製剤の現在の製造工程では、ヒトパルボウイルス B19 等のウイルスを完全に不活化・除去することが困難であるため、本剤の投与によりその感染の可能性を否定できないので、投与後の経過を十分に観察すること。
6. 妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には、治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。〔妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎

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報告企業の意見	今後の対応	
ヒトパルボウイルスB19（human parvovirus B19：B19）は、脂質エンベロープを持たない極めて小さな（約20～26nm）DNAウイルスで、輸血や血漿分画製剤による伝播が報告されている。他のウイルスに比べて、血漿分画製剤の製造工程での不活化・除去が困難であり、本ウイルスの伝播リスクを完全に否定することはできないため、1996年11月より、使用上の注意にB19についての記載を行い注意喚起を図ってきた。万一、原料血漿にB19が混入したとしても、CPVをモデルウイルスとしたウイルスクリアランス試験成績及びB19を用いた不活化･除去試験の結果から、本剤の製造工程において十分に不活化・除去されると考えている。なお、原料血漿へのB19混入量低減のため、B19-ミニプールNATが米国の原料供給元で行われている。	本報告は本剤の安全性に影響を与えないものと考えるので、特段の措置はとらない。	児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。〕

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Caution in evaluation of removal of virus by filtration: Misinterpretation due to detection of viral genome fragments by PCR

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ABSTRACT

The testing of biological products at different stages of the manufacturing process currently involves quantitative polymerase chain reaction (Q-PCR)-based assays. Q-PCR techniques are able to detect not only the viral genome in viral particles but also fragments of degraded genome in samples. The ability of 15 and 19-nm filters to remove viruses was examined by conducting infectivity assays and Q-PCR assays using parvovirus B19 (B19), one of the smallest non-enveloped viruses. Although the filtered samples showed no infectivity, viral DNA was detected by Q-PCR. Interestingly, approximately 90% of the total viral genome in 15-nm filtrates had a detectable size of less than 0.5 kb by the Q-PCR and as a consequence reduction factors were underestimated using Q-PCR. The reduction factors using Q-PCR might be underestimated due to the presence of a large amount of free B19 DNA which shows no infectivity in the tested filtrates. Therefore, the results of Q-PCR should be interpreted with caution. The careful design of primers is needed to eliminate amplification from fragments of viral DNA by Q-PCR.

1. Introduction

The implementation of polymerase chain reaction (PCR)-based testing for viral genomes in donated plasma (usually in mini-pools) has contributed to reductions in viral load during the manufacture of plasma pools (Roth et al., 2000). Heat treatment which inactivates many classes of virus as well as solvent/detergent treatment which only inactivates enveloped viruses, and also several steps in the manufacture of plasma products have helped to reduce the risk of potential transmission of viruses such as hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV). Filtration using membranes with a pore size of only a few nanometers (nm), often called nano-filtration, is one of the most effective means of removing non-enveloped viruses such as parvovirus B19 (B19) and hepatitis A virus (HAV), as well as enveloped viruses. Nanofiltration has been applied to the manufacturing of various plasma products (Burnouf and Radosevich, 2000, 2003). Generally, infectivity assays have been implemented for the quantitation of viruses in samples according to guidelines for virus clearance studies (ICH, 1997; EMEA, 1996; MHLW Japan, 1999). Recently, quantitative PCR (Q-PCR) techniques which demonstrate a high level of precision and fast throughput have been introduced to evaluate the removal of viruses through filtration (Lovatt, 2002).

In this study, infectivity and Q-PCR assays were compared for the evaluation of virus removal over 15 and 19 nm filters using B19 as model virus. The study revealed the presence of B19 in samples that were found positive by Q-PCR but which was not infectious, implying that this Q-PCR signal could be derived from DNA fragments of the viral genome. Therefore, the data obtained by Q-PCR should be interpreted with caution.

2. Materials and methods

2.1. Viruses

A B19 genotype 1-positive plasma sample (termed F15) was used in these studies. The plasma was collected by a licensed establishment in the USA according to local regulations. The number of genomes in F15 was quantified as 11.2 Log_{10} International Unit (IU) (Saldanha et al., 2002)/mL (Artus™ Parvo B19 TM PCR Kit, Artus, Hamburg, Germany). F15 was also tested for other viral markers,

Abbreviations: B19, parvovirus B19; BMM, Bemberg Microporous Membrane; HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IVIG, intravenous immunoglobulin; LRF, Log_{10} reduction factor; PCR ELISA, polymerase chain reaction enzyme-linked immunosorbent assay; Q-PCR, quantitative polymerase chain reaction.

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according to good manufacturing practices: it tested negative for anti-HIV-1/2 IgG, HBV surface antigen (HBsAg), anti-HCV IgG and anti-B19 IgG in enzyme immunoassays. In addition, HIV-RNA, HAV-RNA, HBV-DNA and HCV-RNA were below the limit of detection using nucleic acid amplification tests. All plasma samples were converted to serum by the addition of a 1:20 volume of 4% $CaCl_2$ followed by incubation at 32 °C for 30 min. Fibrin clots were removed with a Büchner funnel and the samples stored at −80 °C.

2.2. Test materials

Samples collected immediately before the nano-filtration steps in the manufacturing process of antithrombin, haptoglobin and intravenous immunoglobulin (IVIG) products (Neuart®$_{I.V.}$, Haptoglobin$_{I.V.}$ and Venoglobulin®IH 5%$_{I.V.}$, respectively; Benesis Corp., Osaka, Japan) were used. These products have been manufactured using source plasma derived from healthy volunteer donors in Japan. The plasma pools used for manufacture of the products was also tested for the viral markers listed above and all were negative. In addition, B19 virus antigen was screened using a receptor-mediated hemagglutination assay (Sakata et al., 1999).

2.3. Removal of viruses by filters

B19 was purified by ultracentrifugation at 150,000 × *g* for 3 h and the resultant pellet was re-suspended in respective start material for each filtration experiment. The suspensions were sonicated to disperse the virus and passed through a 0.22-μm filter (SLGV033RS, Millipore Corp., Billeria, MA). For IVIG solutions, to ensure the formation of virus–antibody complexed forms, B19 was added to IVIG samples and adjusted to pH 5.5 where a complex of anti-B19 antibody in IVIG with B19 particles forms. The sample was sonicated, passed through a 0.22-μm filter, made up to required volumes for filtration, and adjusted to pH 4.2. The B19-spiked antithrombin sample was subjected to filtration using Planova-15N (Asahi Kasei Medical Corp., Tokyo, Japan, 15 ± 2 nm, 0.001 m^2 module). The B19-spiked haptoglobin and IVIG samples were subjected to filtration using Planova-20N (Asahi Kasei Medical, 19 ± 2 nm, 0.001 m^2 module). Conditions for the filtration mimicked (i.e. appropriately down-scaled) the manufacturing conditions for each product according to published guidelines (ICH, 1997; EMEA, 1996; MHLW Japan, 1999). The filtration of each preparation was performed in two runs.

2.4. Infectivity assay of B19

The infectivity assay for B19 utilised cell culture followed by a PCR detection of spliced mRNA as described previously (Hattori et al., 2007). The infectivity of B19 in the samples was titered using KU812 cells (JCRB0104; Health Science Research Resources Bank, Tokyo, Japan) and subsequently viral mRNA expressed in infected wells was detected by RT-PCR. Briefly, 5.3 Log_{10} cells were inoculated with 0.1 mL of 10-fold serial dilutions of samples and cultured in RPMI-1640 medium containing 10% fetal bovine serum, 6 IU/mL of erythropoietin (Sankyo Co., Ltd., Tokyo, Japan), 100 U/mL of penicillin, 100 μg/mL of streptomycin (Invitrogen Corp., Carlsbad, USA), 1 mL of sodium pyruvate (Invitrogen Corp.), and an ITS-X supplement (Invitrogen Corp.) for 4 days. Wells were monitored for the presence or absence of viral mRNA. The spliced viral mRNA was detected by RT-PCR and agarose gel electrophoresis followed by staining with ethidium bromide. The sense primer was B19-25 (nt 2098–2117; 5′-GTCGGAAGCCCAGTTTCCTC-3′) and the antisense primer was B19-11 (nt 3163–3144; 5′-TGCACCAGTGCTGGCTTCTG-3′), where the nt numbers refer to the Au sequence (GenBank Accession No. M13178). The RT-PCR titre (Log_{10} dilution) was determined by end-point dilution and the result was considered positive when the amount of B19 genome determined with the Artus Kit was more than 5.3 Log_{10} IU/mL (4.3 Log_{10} IU/well). Control runs, with the same experimental conditions but without filtration, were also performed. Cytotoxicity tests were also performed and infectivity was measured in the non-cytotoxic range as described above.

2.5. B19 DNA assays

B19 DNA in samples before and after filtration was extracted with a QIAamp viral RNA mini kit (QIAGEN Inc., Valencia, CA) and quantified using a quantitative PCR assay kit (Artus™ Parvo B19 TM PCR Kit). The kit was used in accordance with the manufacturer's instructions using the Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster, CA). Briefly, the reaction mixture that consisted of 30 μL of Master Mix and 10 μL of purified DNA was brought up to a final volume of 50 μL with distilled water. The reaction profile was as follows: 95 °C for 10 min, then 45 cycles of 95 °C for 15 s and 60 °C for 1 min. A 76-bp region of the B19 genome was amplified and the product was detected by measuring the fluorescence of FAM-labeled probe which hybridized specifically to the amplicon. For the quantitative assay, a full set of five Quantitation Standards served as positive controls and results were expressed in international units (IU) (Saldanha et al., 2002) per milliliter. The range of linearity was from 10 IU to 6 Log_{10} IU/reaction and the detection limit was 4 IU/reaction.

PCR ELISA is a qualitative method of detecting B19 DNA by labeling amplicons with digoxigenin (DIG) (Hattori et al., 2007). The samples were diluted 10-fold and B19 DNA was extracted from the samples using Smitest Ex R&D (Genome Science Laboratories, Aichi, Japan), following the manufacturer's instructions. Five volumes of protease solution were added to 1 volume of sample and incubated at 55 °C for 30 min. After proteolysis, 4 volumes of protein denaturation solution were added, incubation continued at 55 °C for 15 min, and then 8 volumes of isopropanol were added, and the mixture was placed on ice for more than 15 min. After centrifugation at 20,000 × *g* for 20 min, pellets were washed with 70% ethanol twice. The region encoding the B19 V region (376 bp) was amplified using a PCR ELISA (DIG-Labeling) Kit (Roche Diagnostics, Mannheim, Germany) with primers B1 (nt 3187–3206; 5′-CAAAAGCATGTGGAGTGTGAGG-3′) and B2 (nt 3558–3539; 5′-GTGCTGTCAGTAACCTGTAC-3′). The amplicon incorporates the DIG-labeled nucleotide during the PCR. The DIG-labeled PCR product was detected using a PCR ELISA (DIG-Detection) Kit (Roche Diagnostics). The amplicon was denatured and hybridized with a biotin-labeled oligonucleotide, probe B (nt 3310–3339; 5′-TAGCTGCCACAATGCCAGTGGAAAGGAGGC-3′), in which nt numbers refer to the Au sequence (GenBank Accession No. M13178). This hybrid was immobilized on a streptavidin-coated microplate and detected with a peroxidase-conjugated anti-digoxigenin antibody and the colorimetric substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS). The PCR titre was determined by end-point dilution.

2.6. Agarose gel electrophoresis and recovery of DNA from gel

B19 DNA was extracted using Smitest Ex R&D (see above). The pellets were dissolved in 10 μL of 50 mM Tris–HCl (pH 7.5) buffer containing 100 mM NaCl and anealed at 60 °C for 30 min. One microgram of a 1-kb DNA Ladder (Invitrogen, Carlsbad, CA) was added to the extract and an aliquot was subjected to gel electrophoresis with 1.0% SeaKem GTG agarose (Lonza, Rockland, ME). The agarose gel was stained with ethidium bromide and cut into five sections (<0.5, 0.5–1.0, 1.0–2.0, 2.0–4.0 and >4.0 kb). DNA was extracted from each section using a QIAquick Gel Extraction Kit (QIAGEN) in accordance with the manufacturer's instructions.

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Table 1
LRF of B19 infectivity and Q-PCR signals by three filtration processes.

Preparations pore size	Antithrombin 15 nm		Haptoglobin 19 nm		IVIG 19 nm	
Method	Infectivity (NDP)	Q-PCR (Log_{10} IU/mL)	Infectivity (NDP)	Q-PCR (Log_{10} IU/mL)	Infectivity (NDP)	Q-PCR (Log_{10} IU/mL)
Pre	5.0/6.0	9.8/9.8	4.0/4.0	8.7/8.7	<1.0/<1.0	8.8/8.4
Post	<1.0/<1.0	5.0/5.3	<1.0/<1.0	5.3/5.4	<1.0/<1.0	6.3/6.2
LRF	≥4.0/≥5.0	4.8/4.5	≥3.0/≥3.0	3.4/3.3	*/*	2.5/2.2

LRF, log reduction factor; NDP, non-detectable end-point; *, could not be calculated.

Briefly, three volumes of Buffer QG were added to 1 volume of gel and incubated at 50 °C for 10 min. After the gel slice was dissolved completely, one volume of isopropanol was added, and the mixture was placed in a spin column and centrifuged at 17,900 × *g* for 1 min. Next, 0.75 mL of washing Buffer PE was added, and the spin column was centrifuged at 17,900 × *g* for 1 min and then centrifuged once more. To elute DNA, 50 μL of water was placed on the column and centrifuged at 17,900 × *g* for 1 min. The B19 DNA in the extract of each section was quantified with the Artus B19 Kit.

3. Results

3.1. Removal of B19 in three steps

The removal of B19 was examined using both infectivity assays and the amount of viral genome determined by Q-PCR (Table 1). The results were evaluated as a Log_{10} reduction factor (LRF) in three different steps. The infectivity in filtrates of the antithrombin and haptoglobin samples was shown to be below the detection limit whereas the infectivity in the non-filtered control experiment done in parallel with the main filtration experiment was unchanged. No infectivity could be detected in samples of the IVIG solution before the filtration, most likely be derived from interference at low pH and/or by neutralization with anti-B19 IgG (Tsujikawa et al., 2011), because B19 DNA was detected in all the samples by Q-PCR (see Table 1).

3.2. Size distribution of B19 DNA in the filtrates of three different samples

The B19 DNA in the samples before and after filtration of the three plasma preparations was divided into five fractions by genome size using agarose gel electrophoresis. The B19 DNA in each fraction was quantified by Q-PCR and percentages of B19 genome amounts were determined (Fig. 1). The calculated percentage was considered to be only slightly influenced by the rate of recovery from each gel section because the rates were 54–85%. The majority of the fragments of B19 DNA in the three spiked process samples (start material) were the long chain (>4.0 kb fraction) including the full-length genome (approximately 5.6 kb) and less than 0.3% of the short chain (<0.5 kb fraction). In sharp contrast, the proportion of short chain B19 DNA were significantly increased to 84.8/93.3% in the sample after 15 nm filtration of the antithrombin sample, reflecting an enrichment of shorter chain fragments following filtration. The majority of the B19 DNA in the 19-nm filtrates of the haptoglobin and IVIG preparations was less than 1.0 kb. The percentage of the long chain B19 DNA in the antithrombin, haptoglobin, and IVIG sample after filtration was 2.0/0.5, 19.9/24.1, and 4.8/5.9%, respectively.

3.3. Reductions with different B19 DNA fragment sizes

The LRFs in different DNA size fractions in the three filtration steps were calculated from the amount of B19 DNA in each fraction (Table 2). For the 15-nm filtrate of the antithrombin sample and 19-nm filtrate of the haptoglobin sample, LRFs increased in proportion to DNA size and the short chain B19 DNA fragments were also removed to varying extents through the filters. In general, the LRFs in each fraction in the haptoglobin sample were lower than in the antithrombin sample. In the 19-nm filtrates of the IVIG sample, the LRF in the <0.5 kb and 0.5–1.0 kb fractions was approximately zero, and the LRFs in the more than 1.0 kb fractions increased with DNA size. Although the LRFs of the short chain B19 DNA were low, the LRFs of the long chain B19 DNA including full genomes in the

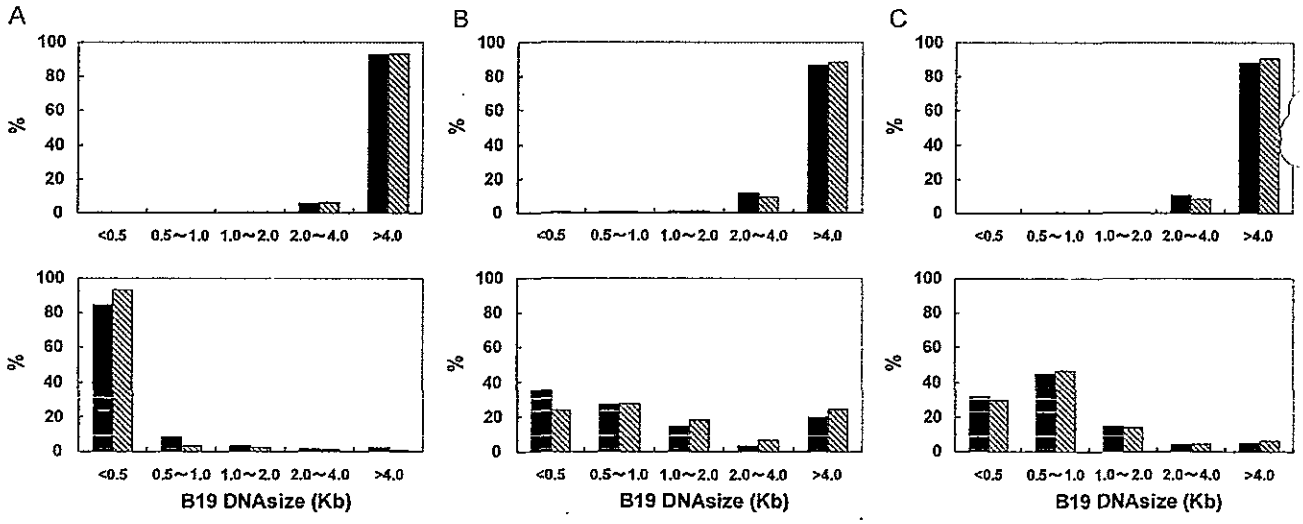

Fig. 1. Relative percentage of B19 genomes in different DNA size fractions in samples before (upper) and after (lower) filtration of three process samples of antithrombin, haptoglobin and IVIG products in duplicate. The 15-nm filtration of antithrombin (A), 19-nm filtration of haptoglobin (B), and 19-nm filtration of IVIG (C) are shown. The black and shaded bars show the percentages of run 1 and run 2, respectively.

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Table 2
Reduction of B19 genomes in different DNA size fractions on three virus removal filtration steps.

	DNA size	<0.5	0.5–1.0	1.0–2.0	2.0–4.0	>4.0 kb
Antithrombin	Pre	7.3/7.0	7.5/7.2	7.5/7.4	8.5/8.5	9.8/9.7
15 nm	Post	4.9/5.2	3.9/3.8	3.5/3.6	3.2/3.3	3.3/3.0
	Reduction	2.4/1.7	3.6/3.4	4.0/3.8	5.3/5.2	6.5/6.7
Haptoglobin	Pre	5.8/6.3	6.5/6.6	6.7/6.9	7.8/7.7	8.7/8.7
19 nm	Post	4.8/4.8	4.7/4.9	4.5/4.7	3.8/4.2	4.6/4.8
	Reduction	1.0/1.4	1.8/1.7	2.3/2.2	4.0/3.4	4.1/3.9
IVIG	Pre	5.7/5.2	6.1/5.8	6.7/6.2	7.8/7.3	8.7/8.3
19 nm	Post	5.8/5.7	6.0/5.9	5.5/5.3	4.9/4.8	5.0/5.0
	Reduction	−0.1/−0.4	0.1/0.0	1.2/0.8	2.9/2.5	3.7/3.4

B19 genome values are shown as Log_{10} IU/mL [Log_{10}(genome amounts in neat × (values in Fig. 1/100))].

antithrombin, haptoglobin and IVIG sample were 6.5/6.7, 4.1/3.9 and 3.7/3.4, respectively.

4. Discussion

The removal and/or inactivation of viruses during the manufacturing of biological products has been evaluated mainly using infectivity assays (ICH, 1997; EMEA, 1996; MHLW Japan, 1999). PCR may be of value in studies of processes which depend on the removal of viruses (EMEA, 1996), particularly where infectivity systems for a particular virus of concern are difficult to perform. Recently, Q-PCR assays were introduced to evaluate the removal of viruses by filtration, because they have a high precision and fast throughput (Lovatt, 2002). In addition, infectivity assays alone are unable to distinguish between removal and inactivation. Therefore, infectivity in combination with Q-PCR assays may provide more valuable information.

In one Q-PCR based study, viral genomes were detected in the sample after filtration, whereas the infectivity was below the detection limit using mouse minute virus and hepatitis A viruses (Kreil et al., 2006). In the present study, the infectivity in the filtrates of three different spiked products were below the detection limit, but the viral genome was detected in all the samples by Q-PCR. The result was positive when the amount of B19 genome determined with the Artus Kit was more than 5.3 Log_{10} IU/mL. The detection limit of the infectivity assay was approximately 4 Log_{10} lower than that of the Q-PCR assay. Therefore, whether the genomes detected by Q-PCR in filtrates are derived from full-genomes in infectious particles or degraded fragments of the genome was examined.

In this study, the size distributions of B19 DNA in filtrates using three process samples were examined. The LRF in each fraction of B19 DNA depended on the DNA fragment size. No reduction was shown in the samples with <1.0 kb of the IVIG filtrate. The degradation may be enhanced by hydrolysis at low pH. Interestingly, it was revealed that the proportion of degraded B19 DNA fragments in the filtrates of the three process samples was enhanced after 15 or 19-nm filtration. Notably, approximately 90% of B19 DNA in the 15-nm filtrate of the antithrombin sample was short chain DNA. Previously, it was reported that the LRF for 15-nm filtration using the antithrombin sample by PCR ELISA was 7.2 (Yunoki et al., 1999). PCR ELISA is a qualitative method for detecting B19 DNA by labeling amplicons with digoxigenin (DIG) (Yunoki et al., 2003). In contrast, the LRF for the same step was 4.8/4.5 (n = 2) by the Q-PCR method in this study. The short chain of B19 DNA in the 15-nm filtrate of the antithrombin sample detected by Q-PCR in this study (A 76 bp), but not by PCR ELISA (A 372 bp), may account for the difference in LRFs observed in the two studies. The size of PCR products obtained with the Artus kit in different fractions has been confirmed as 76 bp by agarose gel electrophoresis (data not shown). The detection limit for the short chain of B19 DNA by PCR ELISA was 2 $Logs_{10}$ lower than that of the long chain (data not shown). The significant difference in LRFs between Q-PCR and PCR ELISA could be explained by the enhanced ability to detect short chain DNA using the Q-PCR assay used in this study. The LRF for long chain B19 DNA in the filtration of the antithrombin sample was approximately 2 $Logs_{10}$ higher than that for un-fractionated samples by Q-PCR. On the other hand, the LRF determined by the PCR ELISA was close to the result of the long chain B19 DNA fraction by Q-PCR. The LRF determined by Q-PCR which can detect short chain DNA may therefore be underestimated, if the LRF from the long chain of B19 DNA reflects the capacity to remove infectious viral particles. A primer set which could amplify the long chain of DNA would therefore provide more relevant results in such instances. Primer sets for use to quantify longer DNA are currently under investigation in our laboratories. A primer set capable of amplifying approximately 1 kb of B19 DNA without a decrease in the detection limit has been obtained, although a primer set able to amplify >2 kb of B19 DNA without a decrease in the detection limit could not be established. Because large amounts of B19 DNA fragments of <1 kb exist in the filtrates of three process samples, a PCR system capable of quantifying approximately 1 kb of DNA might be effective for evaluation of viral removal. Therefore, attention should be paid to designing primers, especially to eliminate underestimation.

In conclusion, Q-PCR assays may be of use to evaluate viral removal, and complement infectivity assays. However, it should be noted that the result obtained by Q-PCR might underestimate the removal capacity of the manufacturing process where degraded fragments of viral genome may serve as targets in the Q-PCR assay. Hence the results of Q-PCR should be interpreted cautiously. Notably, the careful design of primers is needed to eliminate amplification by Q-PCR from viral DNA fragments.

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医薬品　研究報告　調査報告書

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一般的名称	別紙のとおり。	研究報告の公表状況	MMWR.2011;60:1213-1215	公表国 米国	
販売名(企業名)	別紙のとおり。				

研究報告の概要

問題点：米国において、ブタインフルエンザA（H3N2）による熱性呼吸器疾患の患者から2009年に流行したインフルエンザA（H1N1）と考えられるウイルスとのリアソータント株が初めて同定された。

米国において、ブタインフルエンザA（H3N2）による熱性呼吸器疾患の患者2名から2009年に流行したインフルエンザA（H1N1）と考えられるウイルスとのリアソータント株が初めて同定された。2名とも入院後、回復した。1名はインディアナ州の男児で、ブタへの直接接触は確認されていないが、発症の2日前にブタと直接接触したケアワーカーと接触していた。男児の家族、男児との濃厚接触者、ケアワーカー、ケアワーカーの家族及びケアワーカーとの濃厚接触者に呼吸器系疾患に罹患した者は確認されなかった。もう1名はペンシルバニア州の女児で、農業祭におけるブタ及び他の動物への直接接触が確認された。女児の家族及び女児との濃厚接触者に罹患した者は確認されなかったが、農業祭参加者については調査中である。

2名の間に疫学的な関連は特定されておらず、現時点でヒトの追加症例は確認されていない。2名とも発症後の検査でブタインフルエンザA（H3N2）と同定されていたが、その後の全塩基配列決定で2009年に流行したインフルエンザA（H1N1）のものと考えられる配列が判明し、リアソータント株であることが示された。

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

使用上の注意記載状況・その他参考事項等

記載なし。

10

MedDRA/J ver.14.0

別紙

一般的名称	①人血清アルブミン、②人血清アルブミン、③人血清アルブミン*、④人免役グロブリン、⑤人免役グロブリン、⑥人免役グロブリン、⑦乾燥ペプシン処理人免疫グロブリン、⑧乾燥ペプシン処理人免疫グロブリン、⑨乾燥スルホ化人免疫グロブリン、⑩乾燥スルホ化人免疫グロブリン、⑪乾燥スルホ化人免疫グロブリン、⑫乾燥スルホ化人免疫グロブリン、⑬乾燥スルホ化人免疫グロブリン、⑭乾燥スルホ化人免疫グロブリン*、⑮乾燥濃縮人活性化プロテインC、⑯乾燥濃縮人血液凝固第Ⅷ因子、⑰乾燥濃縮人血液凝固第Ⅷ因子、⑱乾燥濃縮人血液凝固第Ⅷ因子、⑲乾燥濃縮人血液凝固第Ⅷ因子、⑳乾燥濃縮人血液凝固第Ⅸ因子、㉑乾燥濃縮人血液凝固第Ⅸ因子、㉒乾燥濃縮人血液凝固第Ⅸ因子、㉓乾燥濃縮人血液凝固第Ⅸ因子、㉔乾燥濃縮人血液凝固第Ⅸ因子、㉕乾燥濃縮人血液凝固第Ⅸ因子、㉖乾燥濃縮人血液凝固第Ⅸ因子、㉗乾燥抗破傷風人免疫グロブリン、㉘乾燥抗破傷風人免疫グロブリン、㉙抗HBs人免疫グロブリン、㉚抗HBs人免疫グロブリン、㉛トロンビン、㉜フィブリノゲン加第ⅩⅢ因子、㉝フィブリノゲン加第ⅩⅢ因子、㉞乾燥濃縮人アンチトロンビンⅢ、㉟乾燥濃縮人アンチトロンビンⅢ、㊱ヒスタミン加人免疫グロブリン製剤、㊲ヒスタミン加人免疫グロブリン製剤、㊳人血清アルブミン*、㊴人血清アルブミン*、㊵乾燥ペプシン処理人免役グロブリン*、㊶乾燥濃縮人アンチトロンビンⅢ
販売名(企業名)	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤ガンマーグロブリン筋注450mg/3mL「化血研」、⑥ガンマーグロブリン筋注1500mg/10mL「化血研」、⑦献血静注グロブリン“化血研”、⑧献血グロブリン注射用2500mg「化血研」、⑨献血ベニロンーⅠ、⑩献血ベニロンーⅠ静注用500mg、⑪献血ベニロンーⅠ静注用1000mg、⑫献血ベニロンーⅠ静注用2500mg、⑬献血ベニロンーⅠ静注用5000mg、⑭ベニロン*、⑮注射用アナクトC2,500単位、⑯コンファクトF、⑰コンファクトF注射用250、⑱コンファクトF注射用500、⑲コンファクトF注射用1000、⑳ノバクトM、㉑ノバクトM注射用250、㉒ノバクトM注射用500、㉓ノバクトM注射用1000、㉔ノバクトM静注用400単位、㉕ノバクトM静注用800単位、㉖ノバクトM静注用1600単位、㉗テタノセーラ、㉘テタノセーラ筋注用250単位、㉙ヘパトセーラ、㉚ヘパトセーラ筋注200単位/mL、㉛トロンビン“化血研”、㉜ボルヒール、㉝ボルヒール組織接着用、㉞アンスロビンP、㉟アンスロビンP500注射用、㊱ヒスタグロビン、㊲ヒスタグロビン皮下注用、㊳アルブミン20%化血研*、㊴アルブミン5%化血研*、㊵静注グロブリン*、㊶アンスロビンP1500注射用
報告企業の意見	インフルエンザウイルスは70～120nmの球形または多形性で、核酸は8本の分節状マイナス一本鎖RNA、エンベロープを有し、エンベロープの表面に存在する赤血球凝集素(HA)とノイラミダーゼ(NA)の抗原性により16種類のHA亜型および9種類のNA亜型に分類される。 今回の報告はブタインフルエンザA(H3N2)と2009年に流行したインフルエンザA(H1N1)とのリアソータント株を示唆する初めての報告であるが、ヒトに対し高病原性であるような情報は示されていない。 上記製剤の製造工程には、冷アルコール分画工程、ウイルス除去膜ろ過工程、加熱工程等の原理の異なるウイルスクリアランス工程が導入されており、各工程のウイルスクリアランス効果は「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン(医薬発第1047号、平成11年8月30日)」に基づく、モデルウイルスを用いたウイルスプロセスバリデーションにより確認されている。今回報告したブタインフルエンザA(H3N2)ウイルスのモデルウイルスには、エンベロープの有無、核酸の種類等から、ウシウイルス性下痢ウイルス(BVDV)が該当すると考えられるが、上記工程のBVDVクリアランス効果については上記バリデーションにより確認されている。また、これまでに上記製剤によるブタインフルエンザA(H3N2)とインフルエンザA(H1N1)とのリアソータント株への感染報告例は無い。 以上の点から、上記製剤はインフルエンザに対する安全性を確保していると考える。

*:現在製造を行っていない

Morbidity and Mortality Weekly Report

Swine-Origin Influenza A (H3N2) Virus Infection in Two Children — Indiana and Pennsylvania, July–August 2011

On September 2, 2011, this report was posted as an MMWR *Early Release on the* MMWR *website (http://www.cdc.gov/mmwr).*

Influenza A viruses are endemic in many animal species, including humans, swine, and wild birds, and sporadic cases of transmission of influenza A viruses between humans and animals do occur, including human infections with avian-origin influenza A viruses (i.e., H5N1 and H7N7) and swine-origin influenza A viruses (i.e., H1N1, H1N2, and H3N2) (*1*). Genetic analysis can distinguish animal origin influenza viruses from the seasonal human influenza viruses that circulate widely and cause annual epidemics. This report describes two cases of febrile respiratory illness caused by swine-origin influenza A (H3N2) viruses identified on August 19 and August 26, 2011, and the current investigations. No epidemiologic link between the two cases has been identified, and although investigations are ongoing, no additional confirmed human infections with this virus have been detected. These viruses are similar to eight other swine-origin influenza A (H3N2) viruses identified from previous human infections over the past 2 years, but are unique in that one of the eight gene segments (matrix [M] gene) is from the 2009 influenza A (H1N1) virus. The acquisition of the M gene in these two swine-origin influenza A (H3N2) viruses indicates that they are "reassortants" because they contain genes of the swine-origin influenza A (H3N2) virus circulating in North American pigs since 1998 (*2*) and the 2009 influenza A (H1N1) virus that might have been transmitted to pigs from humans during the 2009 H1N1 pandemic. However, reassortments of the 2009 influenza A (H1N1) virus with other swine influenza A viruses have been reported previously in swine (*3*). Clinicians who suspect influenza virus infection in humans with recent exposure to swine should obtain a nasopharyngeal swab from the patient for timely diagnosis at a state public health laboratory and consider empiric neuraminidase inhibitor antiviral treatment to quickly limit potential human transmission (*4*).

Case Reports

Patient A. On August 17, 2011, CDC was notified by the Indiana State Department of Health Laboratories of a suspected case of swine-origin influenza A (H3N2) infection in a boy aged <5 years. The boy, who had received influenza vaccine in September 2010, experienced onset of fever, cough, shortness of breath, diarrhea, and sore throat on July 23, 2011. He was brought to a local emergency department (ED) where a respiratory specimen later tested positive for influenza A (H3). The boy was discharged home, but was not treated with influenza antiviral medications. He has multiple chronic health conditions, returned to the ED on July 24, 2011, and was hospitalized for treatment of those health problems, which had worsened. The boy was discharged home on July 27, 2011, and has since recovered from this illness. As part of routine CDC-supported influenza surveillance, the respiratory specimen collected on July 24, 2011, was forwarded to the Indiana State Department of Health Laboratories, where polymerase chain reaction (PCR) testing identified a suspect swine-origin influenza A (H3N2) virus on August 17, 2011. The specimen was forwarded to CDC where the findings were confirmed through genome sequencing on August 19, 2011.

No direct exposure to swine was identified for this child; however, a caretaker reported direct contact with asymptomatic swine in the weeks before the boy's illness onset and provided care to the child 2 days before illness onset. No respiratory illness was identified in any of the child's family or close contacts, the boy's caretaker, or in the family or contacts of the caretaker.

Patient B. On August 24, 2011, CDC was notified by the Pennsylvania Department of Health of a suspected case of swine-origin influenza A (H3N2) virus infection in a girl aged <5 years. The girl, who had received influenza vaccine in September 2010, experienced acute onset of fever, nonproductive cough, and lethargy on August 20, 2011. She was brought to a local hospital ED where a nasopharyngeal swab tested positive for influenza A by rapid influenza diagnostic test. She was not treated with influenza antiviral medications and was discharged home the same day. The girl has completely recovered from this illness.

A nasopharyngeal swab and nasal wash specimen were obtained at the ED and forwarded to the Pennsylvania State Department of Health Bureau of Laboratories for additional testing as part of routine CDC-supported influenza surveillance. On August 23, 2011, the state public health laboratory identified a suspected swine-origin influenza A (H3N2) virus by PCR testing, and both specimens were forwarded to CDC. On August 26, 2011, genome sequencing confirmed the virus as swine-origin influenza A (H3N2). On August 16, 2011, the girl was reported to have visited an agricultural fair where she had direct exposure to swine and other animals. No additional illness in the girl's family or close contacts has been identified, but illness in other fair attendees

continues to be investigated. No additional confirmed swine-origin influenza virus infections have been identified thus far.

Epidemiologic and Laboratory Investigations

As of September 2, 2011, no epidemiologic link between patients A and B had been identified, and no additional cases of confirmed infection with the identified strain of swine-origin influenza A (H3N2) virus had been identified. Surveillance data from both states showed low levels of influenza activity at the time of both patients' illnesses. Case and contact investigations by the county and state human and animal health agencies in Indiana and Pennsylvania are ongoing, and enhanced surveillance for additional human cases is being implemented in both states.

Preliminary genetic characterization of these two influenza viruses has identified them as swine-origin influenza A (H3N2) viruses. Full genome sequences have been posted to publicly available web sites. The viruses are similar, but not identical to each other. Seven of the eight gene segments, including the hemagglutinin (HA) and neuraminidase (NA) genes, are similar to those of swine H3N2 influenza viruses circulating among U.S. pigs since 1998 (*2*) and previously identified in the eight other sporadic cases of human infection with swine-origin influenza A (H3N2) viruses in the United States since 2009.* The one notable difference from the viruses previously identified in human infections with swine-origin influenza A (H3N2) virus is that these two viruses have a matrix (M) gene acquired from the 2009 influenza A (H1N1) virus, replacing the classical swine M gene present in the prior eight swine-origin influenza A (H3N2) virus infections in humans.

Although reassortment between swine influenza and 2009 influenza A (H1N1) viruses has been reported in pigs in the United States (*3*), this particular genetic combination of swine influenza virus segments is unique and has not been reported previously in either swine or humans, based on a review of influenza genomic sequences publicly available in GenBank.† Analysis of data submitted to GenBank via the U.S. Department of Agriculture (USDA) Swine Influenza Virus Surveillance Program subsequent to this case identified two additional influenza A (H3N2) isolates from swine containing the M gene from the 2009 influenza A (H1N1) virus. Genome sequencing is underway to completely characterize the genetic composition of these two swine influenza isolates. (USDA Agricultural Research Service and USDA Animal and Plant Health Inspection Service, unpublished data, 2011).

The viruses in these two patients are resistant to amantadine and rimantadine, but are susceptible to the neuraminidase inhibitor drugs oseltamivir and zanamivir. Because these viruses carry a unique combination of genes, no information currently is available regarding the capacity of this virus to transmit efficiently in swine, humans, or between swine and humans.

Reported by

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Editorial Note

To detect human infections with animal influenza viruses more effectively, CDC and state and local health departments have strengthened laboratory and epidemiologic procedures to promptly detect sporadic cases such as these. Since 2005, state public health laboratories have had the capability to detect non-human origin–influenza A viruses by PCR testing. From 2005 to 2007, CDC received reports of approximately one human infection with a swine-origin influenza virus each year. In 2007, human infection with a novel influenza A virus, including swine-origin influenza virus infections, became a nationally notifiable condition. Since that time, CDC has received approximately three to five reports a year of human infections with swine-origin influenza viruses. The recent increase in reporting might be, in part, a result of increased influenza testing capabilities in public health laboratories that allows for identification of human and swine-origin influenza viruses, but genetic changes in swine influenza viruses and other factors also might be contributing to this increase (*2,5,6*). During December 2005–November 2010, before the two cases described in this report, 21 cases of human infection with swine-origin influenza were reported (12 cases with swine-origin influenza A (H1N1) virus infection, eight cases with swine-origin influenza A (H3N2) virus infection, and one case

*Additional information is available at http://www.cdc.gov/flu/weekly/pastreports.htm.

†Available at http://www.ncbi.nlm.nih.gov/genbank.

What is already known on this topic?

During December 2005–November 2010, 21 cases of human infection with swine-origin influenza were reported, including 12 cases with swine-origin influenza A (H1N1) virus infection, eight cases with swine-origin influenza A (H3N2) virus infection, and one case with swine-origin influenza A (H1N2) virus infection.

What is added by this report?

This report describes two cases of febrile respiratory illness caused by swine-origin influenza A (H3N2) viruses identified on August 19 and August 26, 2011. The viruses identified in these cases are unique in that one of the eight gene segments (matrix [M] gene) is from the 2009 influenza A (H1N1) virus.

What are the implications for public health practice?

Non-human influenza virus infections rarely result in human-to-human transmission, but the implications of sustained ongoing transmission between humans is potentially severe; therefore, prompt and thorough identification and investigation of these sporadic human infections with non-human influenza viruses are needed to reduce the risk for sustained transmission.

with swine-origin influenza A (H1N2) virus infection). Six of these 21 cases occurred in patients who reported direct exposure to pigs; 12 patients reported being near pigs; human-to-human transmission was suspected in two cases after epidemiologic investigations revealed no reported contact with swine in either case, but contact with ill persons who reported swine exposure was the suspected source of infection; the exposure in one case was unknown (*7*) (CDC, unpublished data; 2011). Although the vast majority of human infections with animal influenza viruses do not result in human-to-human transmission (*8,9*), each case should be investigated fully to ascertain whether these viruses are transmitted among humans and to limit further exposure of humans to infected animals, if infected animals are identified. Such investigations require close collaboration between CDC, state and local public health officials, and animal health officials.

The lack of known direct exposure to pigs in one of the two cases described in this report suggests the possibility that limited human-to-human transmission of this influenza virus occurred. Likely transmission of swine-origin influenza A (H3N2) virus from close contact with an infected person has been observed in past investigations of human infections with swine-origin influenza A virus, but has not resulted in sustained human-to-human transmission. Preliminary evidence from the investigation of the Indiana case shows no ongoing transmission. No influenza illness has been identified, but if additional chains of transmission are identified, rapid intervention is warranted to try to prevent further spread of the virus. Clinicians should consider swine-origin influenza A virus infection as well as seasonal influenza virus infections in the differential diagnosis of patients with febrile respiratory illness who have been near pigs. Clinicians who suspect influenza virus infection in humans with recent exposure to swine should obtain a nasopharyngeal swab from the patient, place the swab in a viral transport medium, contact their state or local health department to facilitate transport and timely diagnosis at a state public health laboratory, and consider empiric neuraminidase inhibitor antiviral treatment (*4*). CDC requests that state public health laboratories send all suspected swine-origin influenza A specimens to the CDC, Influenza Division, Virus Surveillance and Diagnostics Branch Laboratory.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	Sobata R, Matsumoto C, Igarashi M, Uchida S, Momose S, Hino S, Satake M, Tadokoro K. Transfusion. 2011 Sep;51(9):1949-56. doi: 10.1111/j.1537-2995.2011.03109.x. Epub 2011 Mar 17.	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			日本

研究報告の概要

○潜伏期間と推定される時期に献血した献血者に、新型インフルエンザ(パンデミック[H1N1]2009)血症は認められなかった

背景: 2009年春、新種のブタ由来インフルエンザA(H1N1)ウイルスが出現し、世界中で流行した。輸血由来と確認されたインフルエンザは報告されていないが、パンデミック(H1N1)2009の大流行は血液製剤の安全性に関して深刻な懸念を引き起こした。日本赤十字社血液センターではパンデミック(H1N1)2009感染疑いのある献血者から得られた血液製剤の供給を中止した。輸血によるパンデミック(H1N1)2009感染のリスクを調査するため、核酸増幅技術を用いて当該製品中のウイルス遺伝子検査を行った。

研究デザインと方法: 2009年6月から12月に、献血後7日以内にパンデミック(H1N1)2009と診断されたか強く疑われた579人の献血者から血液が採取され血液製剤が製造された。ウイルスRNAは血漿製剤と赤血球製剤から抽出し、パンデミック(H1N1)2009ウイルスの赤血球凝集素とマトリックス遺伝子のリアルタイムRT-PCRを実施した。

結果: 579人の献血者から計565の血漿製剤と413の赤血球製剤が得られた。579人の献血者のどのサンプルからもパンデミック(H1N1)2009のウイルスRNAは検出されなかった。

結論: 潜伏期間に献血したと思われる579人の献血者の中にパンデミック(H1N1)2009ウイルスが検出された者は1人もいなかった。輸血によるパンデミック(H1N1)2009の感染リスクは極めて低いと考えられる。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
パンデミック(H1N1)2009ウイルスの潜伏期間に献血をしたと思われる献血者の血液を調査したところ、ウイルスが検出された者は1人もおらず、輸血によるパンデミック(H1N1)2009ウイルス感染のリスクは極めて低いことが示唆されたとの報告である。	日本赤十字社では、問診で発熱などの体調不良者を献血不適としている。また、平成21年5月18日付薬食血発第0518001号「新型インフルエンザの国内発生に係る血液製剤の安全性確保について」に基づき、新型インフルエンザの患者又は罹患の疑いのある患者と7日以内に濃厚な接触があった人の献血を制限するほか、献血後に新型インフルエンザと診断された場合には当該製剤の回収と医療機関への情報提供を行うこととしていたが、平成21年度第4回血液事業部会運営委員会において、献血血液を通じて新型インフルエンザに感染する可能性が極めて低いことが示されたことから、平成22年3月5日に廃止された。今後も引き続き情報の収集に努める。

No viremia of pandemic (H1N1) 2009 was demonstrated in blood donors who had donated blood during the probable incubation period

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BACKGROUND: In the spring of 2009, the novel swine-origin influenza A (pandemic [H1N1] 2009) virus emerged and spread globally. Although no established cases of transfusion-transmitted influenza have been reported, the widespread outbreak of pandemic (H1N1) 2009 caused serious concern regarding the safety of blood products. The Japanese Red Cross Blood Centers have intercepted blood products with accompanying postdonation information indicating possible pandemic (H1N1) 2009 infection. To study the risk of transmission of pandemic (H1N1) 2009 by blood transfusion, we searched for the viral genome in such products using nucleic acid amplification technology.

STUDY DESIGN AND METHODS: Between June and December 2009, blood components were collected from 579 blood donors who were diagnosed as or strongly suspected of having pandemic (H1N1) 2009 within 7 days after donation. Viral RNA was extracted from plasma and red blood cell (RBC) products, and RNA samples were subjected to real-time reverse transcription–polymerase chain reaction of the hemagglutinin and matrix genes of the pandemic (H1N1) 2009 virus.

RESULTS: A total of 565 plasma and 413 RBC products from the 579 blood donors were available. No viral RNA of the pandemic (H1N1) 2009 was detected in any of the blood samples from the 579 blood donors.

CONCLUSION: No viremia of pandemic (H1N1) 2009 was demonstrated in any of the 579 blood donors who had most likely donated blood during the incubation period. It is considered that the risk of transmitting pandemic (H1N1) 2009 by blood transfusion is extremely low.

The novel swine-origin influenza A (pandemic [H1N1] 2009) virus was a triple-reassortant swine influenza virus that contains genes from human, swine, and avian influenza A viruses.[1-3] The pandemic (H1N1) 2009 virus emerged in early 2009 in Mexico and the United States[4,5] and rapidly spread worldwide including Japan[6] via human-to-human transmission because most people have no immunity to this new virus. Although no established cases of transfusion-transmitted influenza have been recognized and reported, the apparently high virulence reported in Mexico[7] raised a serious concern regarding the safety of blood products.

A few studies in the 1960s and 1970s have shown the viremia of seasonal influenza. Most data were obtained from blood samples that were collected after the onset of symptoms.[8-11] Only one instance of the detection of the virus in blood during the incubation period has been reported, but no virus has been detected from blood from the same individual at the onset of symptoms.[11] In recent reports, no viremia of seasonal influenza has been demonstrated.[12,13] Most studies have, thus, failed to demonstrate viremia in blood samples, but this is not unexpected considering that influenza is essentially a respiratory tract

ABBREVIATIONS: HA = hemagglutinin; JRCBSs = Japanese Red Cross Blood Centers; M gene = matrix gene; NIID = Japanese National Institute of Infectious Diseases; PDI = postdonation information.

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infection. There are almost no data on the viremia of pandemic (H1N1) 2009.

We previously reported that there was no viremia detected using nucleic acid amplification technology (NAT) in the blood samples obtained from 96 blood donors who showed symptoms of influenza within 7 days after the donation.[14] In this study, we report the result of our extended study in which 579 blood products from blood donors who were in the same presymptomatic period, as described above, were examined, together with the detailed profiles of such blood donors and the sensitivity of NAT used.

MATERIALS AND METHODS

Collection of blood

In May 2009, when the emergence of pandemic (H1N1) 2009 was confirmed in Japan, the Japanese Red Cross Blood Centers (JRCBCs) implemented the following measures to ensure the safety of blood products, in line with the notification from the Japanese Ministry of Health, Labour and Welfare: screening of blood donors for fever, refusing donation from blood donors who had returned to Japan from abroad within 4 weeks, and refusing donation from donors who had close contact with a patient suspected of having pandemic (H1N1) 2009 within 7 days.

From May 2009 to March 2010, the JRCBCs asked blood donors to provide postdonation information (PDI) on diagnosis of pandemic (H1N1) 2009 in addition to the usual information required. To acquire the PDI, the JRCBCs distributed handbills and explained to all blood donors in all blood collection sites: if they had any symptom of influenza within 7 days after blood donation, and it was diagnosed as pandemic (H1N1) 2009 in medical institutions, they were requested to contact the JRCBCs. If the PDI indicated a possible pandemic (H1N1) 2009 infection after the donation was given to the JRCBCs, the supply of blood products from such blood donors was stopped or withdrawn. These blood products collected from June to December 2009 were used for this study. After the blood products were collected from the inventory or retrieved from medical institutions, they were stored at –20°C in aliquots until use. The time lags between the donation and collection of the blood products for this study were 2 to 30 (mean, 10.3) days for plasma samples and 2 to 18 (mean, 7.8) days for red blood cell (RBC) samples. From the time of donation to collection, these blood products were stored at the stipulated temperatures (plasma, –20°C; RBCs, 4°C). Informed consent to tests for infection was obtained from all blood donors at the blood collection sites.

All blood donors in this study were diagnosed as having pandemic (H1N1) 2009 at a medical institution within 7 days after donation. These blood donors were classified into laboratory-confirmed cases and suspected cases on the basis of the confirmation standard for the diagnosis of pandemic (H1N1) 2009 infection. Laboratory-confirmed cases refer to those diagnosed as having pandemic (H1N1) 2009 by the reverse transcription–polymerase chain reaction (RT-PCR) using respiratory specimens in public health institutes. Suspected cases refer to those diagnosed as having pandemic (H1N1) 2009 infection by the rapid diagnostic kits for influenza A infection or on the basis of the symptoms of influenza-like illness such as fever and acute respiratory symptoms, without performing RT-PCR.

According to the Infectious Agents Surveillance Report published by the Japanese National Institute of Infectious Diseases (NIID), the pandemic (H1N1) 2009 virus dominated 99% of the influenza viruses isolated or detected from the cases of influenza-like illness during the study period from June to December 2009.[15] Therefore, suspected cases in this study were expected to be cases of either pandemic (H1N1) 2009 or noninfluenza illness, with negligible possibility of seasonal influenza.

Evaluation of NAT detection sensitivity

NAT detection sensitivity was evaluated by spiking experiments using virus particles of the pandemic (H1N1) 2009 virus (A/California/04/2009 [H1N1]) contained in the viral culture supernatant donated by NIID. The viral genome copy number of the culture supernatant was determined by quantitative RT-PCR, using synthesized RNA molecules of the matrix (M) gene as standards. The synthesized RNA was obtained from the cloned M gene inserted into plasmid DNA (TOPO TA cloning kit, Invitrogen, Carlsbad, CA) by transcription using T7 RNA polymerase (Roche Diagnostics, Indianapolis, IN). The transcribed RNA was purified using a commercially available kit (RNeasy Plus Mini Kit, Qiagen, Gaithersburg, MD), and its quantity and quality were checked using a capillary electrophoresis system (Agilent 2100 Bioanalyzer and RNA 6000 Nano Kit, Agilent, Santa Clara, CA). A dilution series of the synthesized RNA sample was used to construct a standard curve to estimate the viral genome copy number of the culture supernatant.

The quantified culture supernatant of the pandemic (H1N1) 2009 virus was spiked into plasma and RBC samples from healthy volunteers, at inoculation doses from 20 to 2×10^5 genome equivalents (geq)/mL, and the NAT for the M and hemagglutinin (HA) genes was performed 20 times for each dose. The relationship between NAT positivity and pandemic (H1N1) 2009 virus concentration was analyzed by probit analysis. An input viral genome copy number with a 95% probability of a positive result was used as the detection limit.

NAT for detection of pandemic (H1N1) 2009 virus

Viral RNA was extracted from plasma and packed RBC samples using a kit for automated purification of viral

DNA and RNA (QIAamp Virus Biorobot MDx kit, Qiagen) and a viral nucleic acid purification kit (High Pure Viral Nucleic Acid Large Volume Kit, Roche Diagnostics), respectively. RNA samples were immediately subjected to the real-time RT-PCR of the M and HA genes of influenza A with a sequence detection system (PRISM 7900, Applied Biosystems, Foster City, CA) using an RT-PCR kit (Quanti-Tect Probe, Qiagen). The real-time RT-PCR of the HA gene was designed for the specific detection of the pandemic (H1N1) 2009 virus, whereas the real-time RT-PCR of the M gene was designed for the universal detection of type A influenza viruses. The sequences of the primers and probes used were synthesized in accordance with the protocols developed by NIID.[16] The forward and reverse primers were 5′-CCMAGGTCGAAACGTAYGTTCTCTCTA TC-3′ and 5′-TGACAGRATYGGTCTTGTCTTTAGCCAYTC CA-3′, respectively, for M gene and 5′-AGAAAAGA ATGTAACAGTAACACACTCTGT-3′ and 5′-TGTTTCCACAA TGTARGACCAT-3′, respectively, for HA gene. The TaqMan probes for M and HA genes were 5′-ATYTCGGCT TTGAGGGGGCCTG-3′ and 5′-CAATRTTRCATTTACC-3′, respectively. Each probe was labeled with a reporter dye (FAM) at the 5′ end, a nonfluorescent quencher and a minor groove binder at the 3′ end. Either 200 μL of plasma or 100 μL of RBC samples was used for each test. The real-time RT-PCR conditions comprised a 30-minute RT step at 60°C, a 10-minute initial PCR activation step at 95°C, and 45 amplification cycles at 95°C for 15 seconds and at 60°C for 45 seconds. To assess the analytical accuracy of NAT, a dilution series of pandemic (H1N1) 2009 virus particles in the viral culture supernatant as a positive control, and plasma and RBC samples obtained from healthy volunteers as negative controls were included in each assay.

Lookback investigation for blood recipients

As one of the operations in hemovigilance, JRCBCs have been collecting information on transfusion-transmitted infections and adverse transfusion reactions such as fever, urticaria, pain, nausea, hypotension, anaphylactic reaction or shock, dyspnea, and neuropsychiatric symptoms. If the blood products had already been released when the PDI indicating possible pandemic (H1N1) 2009 infection was acquired, we inform the medical institution of the blood product concerned. Patients who had received transfusion with the blood products involved with the pandemic (H1N1) 2009 infection were observed for influenza-like symptoms such as fever, respiratory symptoms, or systemic inflammatory reactions for a period of 7 days after transfusion.

RESULTS

Characteristics of blood donors

Between June and December 2009, the blood components were collected from 579 blood donors (314 male and 265 female). A total of 366 blood donors (190 male and 176 female) had laboratory-confirmed pandemic (H1N1) 2009 infection, and 213 blood donors (124 male and 89 female) had suspected pandemic (H1N1) 2009 infection.

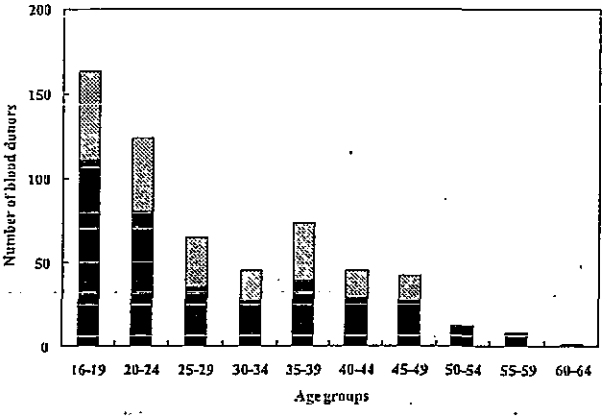

Fig. 1. Age distribution of the blood donors who had symptoms of pandemic (H1N1) 2009 after donation. Laboratory-confirmed cases (black bar) were diagnosed as pandemic (H1N1) 2009 by the RT-PCR method using respiratory specimens. Suspected cases (gray bar) were diagnosed by rapid diagnostic kits or on the basis of the symptoms of influenza-like illness, without performing RT-PCR. Sixty-one percent of the blood donors who showed symptoms of influenza after donation belonged to the young age group and were less than 30 years of age.

The ages of the 579 blood donors are shown in Fig. 1. Sixty-one percent of them were less than 30 years of age. The ratio of the donor number in the 16- to 29-year age group to the 579 blood donors were 2.2 times as high as the ratio of all blood donors in the 16 to 29 age group to the total number of donors who donated in 2009 in Japan. In each age group, there were no significant differences in the ratios between sexes (data not shown).

The time lag between the donation and the onset of influenza symptoms is shown in Fig. 2. Ten (1.7%) blood donors developed symptoms of influenza on the day of the donation, 74 (12.8%) within 1 day, 105 (18.1%) within 2 days, and 132 (22.8%) within 3 days after donation. That is, 321 (55.4%) blood donors showed symptoms of influenza within 3 days after donation.

The Infectious Agents Surveillance Report published by NIID indicated that the fall wave of pandemic (H1N1) 2009 appears to have peaked in late November 2009; this corresponded to the finding that 468 (80.8%) of the 579 blood donors donated between November and December 2009 (data not shown).

Evaluation of NAT detection sensitivity

For the plasma samples, the NAT showed a 100% detection probability for both M and HA genes at more than

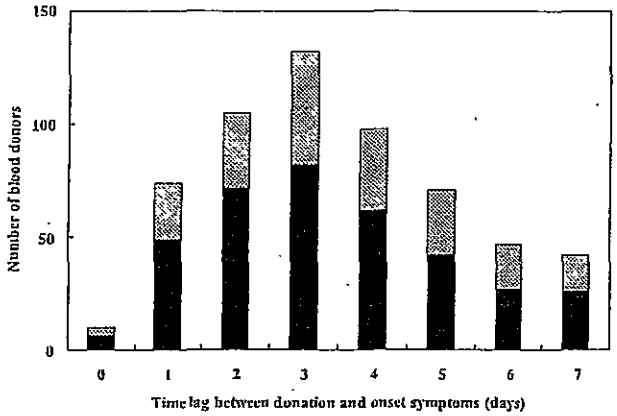

Fig. 2. Time lag between donation and onset of influenza symptoms. Laboratory-confirmed cases (black bar) were diagnosed as pandemic (H1N1) 2009 by the RT-PCR method. Suspected cases (gray bar) were diagnosed by rapid diagnostic kits or on the basis of the symptoms of influenza like illness, without performing RT-PCR. More than half of blood donors (55.4%) showed symptoms of influenza within 3 days after donation. It was speculated that many blood donors gave blood during the incubation period of the pandemic (H1N1) 2009 virus.

2000 geq/mL. The detection probabilities were 85 and 75% at 200 geq/mL and 50 and 15% at 20 geq/mL for the M and HA genes, respectively (Fig. 3A). For the RBC samples, the NAT showed a 100% detection probability for both M and HA genes at more than 20,000 geq/mL. The detection probabilities were 80 and 65% at 2000 geq/mL and 15 and 5% at 200 geq/mL for the M and HA genes, respectively (Fig. 3B).

The 95% detection limit of the NAT in the plasma samples was calculated to be 283 geq/mL (95% confidence interval [CI], 116-3287), corresponding to 57 geq per reaction (95% CI, 23-658) for the M gene, and 528 geq/mL (95% CI, 256-2368), corresponding to 106 geq per reaction (95% CI, 51-474) for the HA gene (Fig. 3A). For the RBC samples, the 95% detection limit of the NAT was calculated to be 3444 geq/mL (95% CI, 1784-12,697), corresponding to 344 geq per reaction (95% CI, 178-1270) for the M gene and 5292 geq/mL (95% CI, 2829-19,911), corresponding to 529 geq per reaction (95% CI, 283-1991) for the HA gene (Fig. 3B).

Detection of pandemic (H1N1) 2009 virus RNA

The NAT was performed using 565 plasma and 413 RBC samples obtained from 579 blood donors who showed symptoms of influenza within 7 days after donation. The samples consisted of 362 plasma and 271 RBC samples from the 366 blood donors who had laboratory-confirmed pandemic (H1N1) 2009 infection and 203 plasma and 142 RBC products from the 213 blood donors who had suspected pandemic (H1N1) 2009 infection. The NAT was performed in duplicate for the M and HA genes of the pandemic (H1N1) 2009 virus in each sample. None of the viral genome of the M or HA gene was detected in any of the plasma samples and RBC samples (Table 1).

Lookback investigation of blood recipients

In the lookback investigation of the donated blood products from the 579 blood donors, it was revealed that 36 platelet (PLT) products and 34 RBC products had already been used for transfusion when the PDI was acquired. Of the 36 blood donors who donated these PLT products, two showed symptoms of influenza on the next day of donation, and 10 and 13 showed symptoms 2 and 3 days after donation, respectively. Of the 34 blood donors who donated these RBC products, two and three showed symptoms of influenza 2 and 3 days after donation, respectively (Fig. 4). Of the blood donors who donated these PLT products and RBC products, 25 and 20 blood donors belonged to laboratory-confirmed cases, respectively.

In the 70 blood recipients who received blood products likely donated during the incubation period of the pandemic (H1N1) 2009 infection, influenza-like symptoms such as fever and acute respiratory symptoms and any transfusion adverse reactions were not observed for a period of 7 days after transfusion.

DISCUSSION

In this study, we examined blood samples from 579 blood donors who were diagnosed as or strongly suspected of having pandemic (H1N1) 2009 infection within 7 days after donation. Sixty-one percent of the blood donors involved in this study belonged to the young age group of less than 30 years of age. The ratio of the young age group to the 579 blood donors was higher than that of this age group to the total number of blood donors who donated in 2009 in Japan. It has been reported that the majority of patients with pandemic (H1N1) 2009 were children and young people.[17,18] According to the reports by the Centers for Disease Control and Prevention in the United States, more than 64% of the pandemic (H1N1) 2009 virus-infected individuals were 5 to 24 years old; only 1% were 65 years of age or older.[17] In this point, pandemic (H1N1) 2009 markedly differs from seasonal influenza. The ratio of the age groups of the blood donor cohort involved in this study reflected the ratio of the age groups of the pandemic (H1N1) 2009 virus-infected individuals reported in Japan and abroad.[17,19]

In this study, 10 blood donors showed symptoms of influenza on the day of the donation, 74 within 1 day, and 105 within 2 days after the donation. The incubation

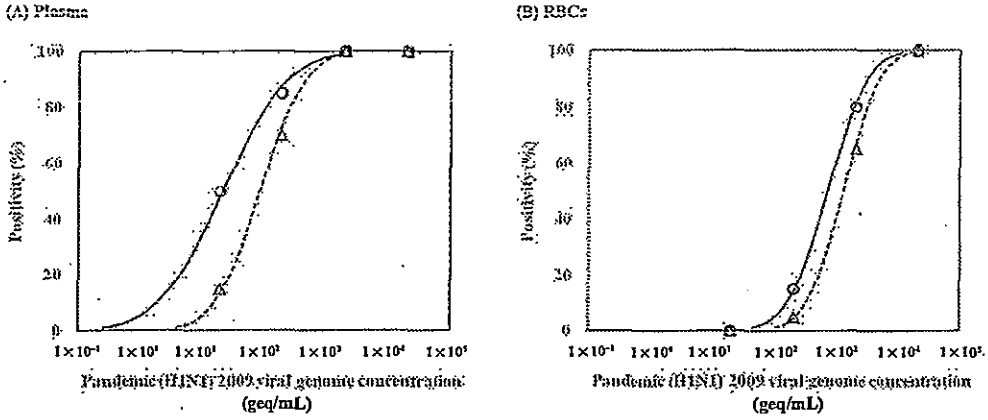

Fig. 3. Sensitivities of the NAT for plasma samples (A) and RBC samples (B). The quantified culture supernatant of the pandemic (H1N1) 2009 virus was spiked into the plasma and RBC samples from healthy volunteers, at doses from 20 to 2×10^5 geq per mL, and the NAT for the M (—) and HA (- - -) genes was performed 20 times for each concentration. The relationship between NAT positivity and pandemic (H1N1) 2009 virus concentration was analyzed by probit analysis. The 95% detection limit of the NAT for the plasma samples was calculated to be 283 geq/mL for the M gene and 528 geq/mL for the HA gene (A). For the RBC samples, the 95% detection limit of the NAT was calculated to be 3444 geq/mL for the M gene and 5292 geq /mL for the HA gene (B).

TABLE 1. Results of detection of pandemic (H1N1) 2009 virus RNA in plasma or RBCs drawn from blood donors who were diagnosed as pandemic (H1N1) 2009 after donation

		Number of samples tested		NAT-positive samples			
				Plasma		RBCs	
Cases	Total number of blood donors	Plasma	RBC	M gene	HA gene	M gene	HA gene
Laboratory-confirmed case*	366	362	271	0	0	0	0
Suspected case†	213	203	142	0	0	0	0
Total	579	565	413	0	0	0	0

* Pandemic (H1N1) 2009 was diagnosed by the RT-PCR method using respiratory specimens.
† Pandemic (H1N1) 2009 was diagnosed by the rapid diagnostic kits for influenza A or on the basis of the symptoms of influenza-like illness, without performing RT-PCR.

period of the pandemic (H1N1) 2009 virus is reported to be 1 to 7 days, with a mean of 2 days.[5,20] Therefore, it is speculated that many of the 579 blood donors gave blood during the incubation period of the pandemic (H1N1) 2009 virus. NAT was performed using specimens of this donated blood. No RNA of the pandemic (H1N1) 2009 virus was detected in any of the blood samples; no viremia of pandemic (H1N1) 2009 before clinical onset was demonstrated. In this study, however, some donors included in the 213 suspected cases were diagnosed by physicians on the basis of the symptoms of influenza-like illness without performing RT-PCR or rapid diagnostic tests. The possibility that donors with noninfluenza illness were not completely excluded from the suspected cases would weaken the power of this study.

Although influenza epidemics occur every winter season, no established cases of transfusion-transmitted influenza have been recognized and reported. A few studies published from the 1960s to the 1970s showed the presence of viremia of seasonal influenza;[8-11] however, among studies published in recent years, no viremia of seasonal influenza has been demonstrated yet.[12,13] In both pandemic (H1N1) 2009 and seasonal influenza virus infections, the peak viral load in the respiratory specimens was observed immediately after the onset of symptoms.[21,22] Although the mean viral load in the respiratory specimens of pandemic (H1N1) 2009 was 1.84×10^8 copies/mL, the virus was detected in none of the blood specimens obtained at the same time.[20] In addition to these data, we detected no viremia of pandemic (H1N1) 2009 in the present study. It thus seems that the occurrence of pandemic (H1N1) 2009 viremia before the onset of illness is extremely low. If there were cases in which influenza infections occurred by blood transfusion, a viremia condition would need to be present during the incubation period in which blood donation was performed. It is thus considered that the risk of the transmission of the pandemic (H1N1) 2009 virus by blood transfusion is extremely low.

The 95% detection limit of NAT for the plasma samples was calculated to be 283 geq/mL for the M gene and 528 geq/mL for the HA gene, whereas that for the RBC

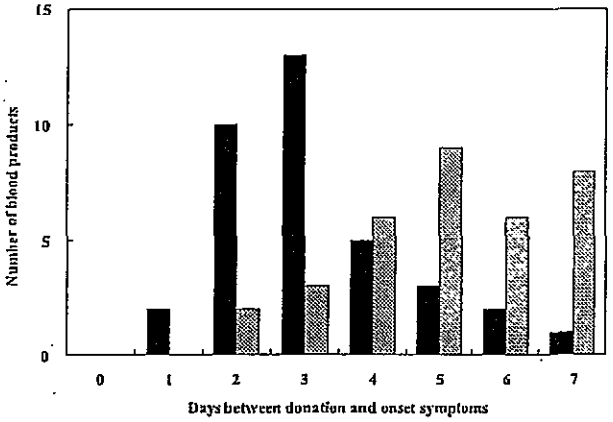

Fig. 4. Time lag between donation of blood implicated in lookback study and onset of symptoms. Thirty-six PLT products (black bar) and 34 RBC products (gray bar) likely donated during the incubation period of the pandemic (H1N1) 2009 had already been used for transfusion to 70 recipients when the PDI was acquired. In the lookback investigation, no influenza-like symptoms or any other observable symptoms were observed in the recipients after transfusion.

samples was calculated to be 3444 geq/mL for the M gene and 5292 geq/mL for the HA gene. The NAT sensitivity for the HA gene was lower than that for the M gene, for both plasma and RBC samples. This difference could be due to the secondary structure of the viral RNA or reverse-transcribed cDNA that might have decreased the sensitivity of the RT-PCR of the HA gene. The lower sensitivity in the packed RBC samples might be caused by the inhibitors of RT-PCR such as hemoglobin that contaminated the RNA solutions obtained from the RBC samples or the insufficient efficiency of the viral RNA extraction. Improved methods will be required to purify viral RNA from RBC samples. If low-level viremia below the NAT detection sensitivity should exist, it would not be detected using our assay.

In the first step in influenza infection and viral replication, influenza viruses bind through the HA transmembrane glycoprotein onto sialic acid residues on the surface of epithelial cells, typical in respiratory organs. After HA is cleaved by a protease, the cells import the virus by endocytosis.[23-25] HA cleavage is required to activate virus infectivity, and the activating proteases are mainly distributed in the respiratory organs and intestine in humans,[24,26] so that it should be difficult for influenza viruses to acquire infectivity in blood. In theory therefore the risk of the direct transmission of influenza via blood is considered to be extremely low. In fact, we showed, in this study, that the transfusion of 36 PLT and 34 RBC products from the blood donors who likely donated during the incubation period of the pandemic (H1N1) 2009 virus caused apparently no transmission of the virus.

The risk of virus infection by blood transfusion has decreased owing to the introduction of a screening test for donated blood, but there is still residual risk caused by pathogens that are excluded from targets of the current screening test or newly spread in humans. The influenza virus is one of the potentially unrecognized pathogens in the blood supply. This study showed that the viremia of pandemic (H1N1) 2009 during the incubation period is highly unlikely to occur and that it does not pose a noticeable risk to the safety of the blood supply. The main infection routes of the influenza virus are droplet infection and contagious infection. During a pandemic, many people are easily infected by the influenza virus. Compared with the risk of infection by the influenza virus via respiratory droplets, the risk of transmission by transfusion is almost negligible. In this point, pandemic (H1N1) 2009 markedly differs from any other currently known viral threats to the blood supply.

Regarding influenza viruses with high pathogenicity, we do not know the risk of their transmission by transfusion. The HA protein of highly pathogenic avian influenza virus can be cleaved by proteases that are produced in many different tissues. As a results, these viruses can replicate in many organs of the bird, not just the respiratory organs.[27] In severe cases of highly pathogenic avian influenza A (H5N1) virus-infected humans, viremia has been reported.[28-30] New studies will be required when a new type of influenza emerges in the future.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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別紙様式第２－１

医薬品　研究報告 調査報告書

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研究報告の概要

ゼノトロピック MLV 関連ウイルス(xenotropic-MLV-related virus：XMRV)を含むマウス白血病ウイルス(MLV)が慢性疲労症候群(CFS)に関連していることが議論されている。
この問題を更に深く検討するため、以前に XMRV/MLV 陽性と報告された被験者 15 例(14 例が CFS 患者)および以前に XMRV/MLV 陰性と判定された健康ドナー15 例から採取した血液サンプルを収集した。
これらのサンプルを二重盲検下で 9 ヵ所の研究所に分配し、XMRV/MLV 核酸、ウイルス複製および抗体を検出する検査を行った。
現在利用可能な XMRV/P-MLV 検査法は、以前に陽性結果を報告した 3 ヵ所の参加研究所が採用している検査法を含め、CFS 患者および対照から採取したサンプルにおいて直接的ウイルスマーカー(RNA、DNA または培養物)あるいは特異的抗体を再現性よく検出できないと結論付けられた。
今回の知見により、現時点では血液提供者への通常のスクリーニングに XMRV/P-MLV に関する検査を実施する正当な理由はないことが示唆された。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
慢性疲労症候群との関連が疑われる XMRV やその他の MLV に対する血液スクリーニングの要否に関する情報であるが、検査の再現性が担保できず、現時点ではスクリーニング項目として採用する正当な理由はないとの情報であった。	今後とも XMRV やその他の MLV に関する安全性情報等に留意していく。

Sciencexpress Report

Failure to Confirm XMRV/MLVs in the Blood of Patients with Chronic Fatigue Syndrome: A Multi-Laboratory Study

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Murine leukemia viruses (MLV), including xenotropic-MLV-related virus (XMRV), have been controversially linked to chronic fatigue syndrome (CFS). To explore this issue in greater depth, we compiled coded replicate samples of blood from 15 subjects previously reported to be XMRV/MLV-positive (14 with CFS) and from 15 healthy donors previously determined to be negative for the viruses. These samples were distributed in a blinded fashion to nine laboratories which performed assays designed to detect XMRV/MLV nucleic acid, virus replication, and antibody. Only two laboratories reported evidence of XMRV/MLVs; however, replicate sample results showed disagreement and reactivity was similar among CFS subjects and negative controls. These results indicate that current assays do not reproducibly detect XMRV/MLV in blood samples and that blood donor screening is not warranted.

Novel murine leukemia virus (MLV)-like sequences were identified in, and implicated as a potential infectious cause of, human prostate cancer in 2006 (*1*). These sequences appeared to be closely related to xenotropic MLV (X-MLV) and were termed X-MLV-related virus or XMRV. In 2009, similar viral sequences were identified in a cohort of patients with chronic fatigue syndrome (CFS) (*2*). In that study XMRV could be directly cultured from both peripheral blood mononuclear cells (PBMC) and plasma from the majority of patients with CFS, and XMRV sequences were detected by PCR and RT-PCR (*2*, *3*). Furthermore, evidence of an immune response to MLVs was observed in patient plasma (*2*, *3*). In an independent study, other patients with CFS were reported to harbor MLV-related virus sequences, but not XMRV, in PBMC and plasma (*4*). These sequences were derived from viruses resembling polytropic MLVs (P-MLV), rather than X-MLV. Importantly, both studies identified XMRV/P-MLV in the majority (67 to 86%) of patients with CFS but also in substantial numbers of healthy controls including blood donors (4-7%) (*2*, *4*).

Subsequent studies cast doubt on the association between XMRV/P-MLVs and CFS, and indeed on the detection of XMRV/P-MLVs in human populations (reviewed in (*5*)). Many, although not all (*6*, *7*), of these negative studies focused on nucleic acid detection and/or serology and did not include cell culture assays for virus (*8–11*). Several additional findings raised uncertainty about the high rates of XMRV/P-MLV in patients with CFS that had been described in the two seminal papers: (i) clinical samples and PCR reagents were found to be contaminated by XMRV and mouse DNA containing endogenous MLVs (*12*); (ii) XMRV and P-MLV lack the sequence diversity that would be expected to arise following transmission, infection, and repeated cycles of replication of a retrovirus in humans (*13*, *14*), and (iii) evidence was presented which strongly suggested that XMRV originated in the early 1990s by recombination of endogenous

MLVs following serial passage of a human prostate xenograft in laboratory mice (*15*). It was postulated that this laboratory passage resulted in the generation of several prostate cancer cell lines harboring integrated XMRV sequences that produced high levels of infectious virions. These XMRV-infected cell lines were subsequently widely disseminated and likely produced inadvertent XMRV contamination of laboratories and reagents (*15*).

We report here the results of a comprehensive study where multiple laboratories analyzed the same blood samples for XMRV/P-MLV. These blood samples, which were drawn from persons who were previously reported to be XMRV- (*2*) or P-MLV-positive (*4*) and from blood donors who previously tested negative for XMRV, were aliquoted into replicate tubes and assembled into coded panels together with replicates of experimentally prepared positive control samples. The testing was performed fully blinded to remove bias. These samples were tested by nine laboratories using highly sensitive and previously validated nucleic acid, serological and culture assays (tables S1 to S5) for XMRV and other MLVs (*16*). The two laboratories that had previously found an association for the MLVs with CFS participated in this study (*2*, *4*). All nine laboratories used XMRV/P-MLV nucleic acid amplification testing (NAT), serological and/or culture assays of their own choosing which were incorporated into parallel or serial testing algorithms to generate final results. The majority of laboratories included assays to detect murine DNA contamination either on all samples or on all NAT positive samples.

Fourteen patients with CFS, together with one person reporting contact with a CFS patient [described in supporting online material (*17*)], all of whom were previously reported to be XMRV/P-MLV-positive by at least one method (table S6) were enrolled into the study at two clinical sites using IRB-approved protocols and consents (referred to as the XMRV/P-MLV cohorts henceforth). Per study protocol, none of the 15 subjects were on antiretrovirals, but several later disclosed that they were taking other antivirals (e.g., valacyclovir) and two were on immunosuppressive medications (the latter are indicated in table S6). In the case of the P-MLV-like viruses described by Lo and colleagues (*4*), only PCR detection had been performed in the original study; four of five patients enrolled into the current study were reported to be P-MLV reactive on the archived samples from the original cohort study and on a second sample collected 15 years later (2010) whereas one patient was PCR-positive only on the original archived sample (*4*). The Whittemore Peterson Institute (WPI) patient cohort was more intensively characterized as positive by PCR, serology and/or culture, although none of the study subjects tested positive in all assays at all time points (table S6).

To minimize introduction of potential contaminants, we took extensive precautionary measures during the collection of specimens and the laboratory processing of blood and preparation of sample aliquots (*17*). Blood specimens were collected by independent phlebotomists, shipped to the central laboratory (*17*), and processed into coded PBMC, plasma and whole blood (WB) aliquots. Similarly, fifteen control specimens from blood donors (n=12) or laboratory controls (n=3) that had been established as negative for XMRV and MLVs by PCR, serology and culture by multiple laboratories, were collected, processed and aliquoted in parallel (*17*). Finally, a separate facility in the central laboratory prepared and characterized stocks of the XMRV-infected human cell line 22Rv1 (*15*, *18*) and supernatant, which were used to spike samples to create a set of low-level positive controls (*17*).

A total of eleven NAT, five serology and three culture assays were performed on the samples (*17*). The WPI laboratory did not report culture assay results because their target cells had become contaminated with mycoplasma. Other than this, all sites reported results on all distributed and coded sample aliquots to the central laboratory. The results were then decoded and compiled into analysis datasets specific to the panels.

Few positive NAT results were reported, other than on the coded spiked positive control replicate aliquots (table 1) (table S7). Six of seven laboratories that performed NAT on three sample types (plasma, PBMC and WB) reported no positive result for coded clinical samples (XMRV/P-MLV cohorts or negative controls), whereas these laboratories detected XMRV in 100% of the spiked controls (table 1). These laboratories included those that employed the most sensitive XMRV/P-MLV assays available, based on our previous blinded analytical sensitivity performance study (*16*). Of particular note, the FDA/Lo laboratory failed to detect MLV-like sequences using the same nested PCR assay as previously published, in either the known negative controls or in the XMRV/P-MLV cohort samples. The samples scored as negative by this laboratory included the replicate samples from five patients with CFS reported as P-MLV positive in their previous study, four of whom had also tested positive on a second specimen collected over a decade after the archived CFS cohort panel (*4*).

The only positive NAT results on some of the replicates from clinical samples were reported by WPI. The WPI assays appeared less sensitive than those used by the other laboratories, based on the fact that only 3 of 5 plasma and 4 of 5 PBMC-spiked positive control replicates were scored as positive by WPI (table 1) (table S7). However, two plasma clinical aliquots were reported as positive in the WPI nested RT-PCR *gag* assay. These samples were from two different negative controls, and only one out of the three replicates was

positive in each case. Sequencing of the excised bands revealed 1-3 base changes compared to XMRV derived from 22Rv1 (supporting online text). A clinical PBMC sample, derived from one of the nine WPI CFS patients, was also positive in WPI's nested *gag* PCR assay. However, only one of two PBMC replicates for this individual was positive, and all replicates of plasma and WB from this patient were reported as negative by WPI. All positive samples tested negative for mouse DNA contamination as assessed by mouse mitochondrial DNA PCR (*4*). Reactivity rates did not significantly differ between samples from negative controls and the XMRV/P-MLV cohorts ($p > 0.05$) (supporting online text, table S10).

In the initial study, Lombardi et al. reported that the most effective and consistent method of determining whether an individual was XMRV-positive was by isolation of replication-competent virus through co-culture of target prostate cell lines with either patient PBMCs or plasma (*2*, *3*). Although culture results were not reported by WPI in the present study, the NCI/Ruscetti laboratory also successfully performed virus culture using both plasma and PBMC in the Lombardi et al. study (*2*, *3*). Additionally, virus culture was performed by the FDA/Hewlett laboratory, which used two methods, one of which (LNCaP cell culture) was established in their laboratory for this study based on WPI procedures and on-site training by the lead investigators from the WPI and NCI/Ruscetti laboratories, and hence viral culture in this laboratory would be expected to have equivalent sensitivity to the culture method used by Lombardi et al. (*17*). Both laboratories successfully detected all five replicates of the spiked positive controls (~10^6 RNA copies/ml). However, while neither of the FDA/Hewlett assays detected confirmed positive cultures in the 30 coded clinical aliquots, the NCI/Ruscetti laboratory reported nine aliquots as positive (table 1, 2). Six of the positive results were from negative control samples (40% positive rate); these six subjects/samples had previously been pedigreed by the same laboratory as culture-negative (*17*). In contrast, only three (20%) of the 15 XMRV/P-MLV-cohort subjects (including ten subjects who had previously been found to be culture-positive by the WPI and NCI/Ruscetti laboratories) tested positive in the coded panel (table S1). There was no significant difference between the rate of reported positive culture results among negative controls and the XMRV/P-MLV cohort subjects (p-value = 0.43, table S8).

Finally, serology was performed by four laboratories (*17*). Although plasma with human antibodies to XMRV/P-MLVs was not available to produce spiked controls for serology, all four laboratories performed their own internal controls (*17*). Three assays --a Western blot test using purified XMRV (CDC) (*19*) and two chemiluminescent immunoassays using recombinant XMRV gp70 and p15E (Abbott Diagnostics) (*20*)-- failed to detect positive results for any of the coded replicates prepared from the 30 clinical samples. A flow cytometry-based serologic assay run by two laboratories (NCI/Ruscetti and WPI), utilizing mouse cells expressing the spleen focus-forming virus (SFFV) envelope as employed in the original Lombardi et al. study, reported a number of positive results on samples from both the XMRV/P-MLV cohorts and the negative-plasma controls. The NCI/Ruscetti laboratory reported 13 positive samples, including eight (53%) from 15 known negatives and five (33%) from 15 XMRV/P-MLV cohort subjects (table 1) (table 2). None of the positive results from the XMRV/P-MLV cohorts or controls were reported for more than one of the uniquely coded replicates, despite the fact that every sample was represented in the panel in duplicate or triplicate (table 2). There was no significant difference between the proportions of negative controls and XMRV/P-MLV cohort subjects identified as serology-positive (p-values >0.20 regardless of how positivity was defined [supporting online text, table S9]).

Among all serologic replicates tested, the WPI detected 22 positives, including 10 reactive results among the negative controls, and six each in the subjects previously reported as positive by WPI and by FDA/Lo (table 1) (table S7). Three of the six known negative controls with a positive serology result had at least two of three replicates positive (table 2). All five patients previously identified as P-MLV positive by FDA/Lo had a replicate called serology positive, but only one had both replicates reported as positive. Similarly for the 10 subjects previously identified as XMRV positive by WPI, four subjects had one of two replicates reported as serology positive, while both replicates from one patient were reported positive (table 2). There was no significant difference in the rates of positive WPI serology results between negative controls and XMRV/P-MLV cohort subjects (p-value = 0.27). There was no statistical agreement between the samples reported as serology positive by the NCI/Ruscetti and WPI laboratories, despite the fact that they used similar assays (supporting online text, tables S9, S10). Kappa values were calculated for each criterion and for all subjects combined using standard procedures (*17*, *21*). The Kappa values for level of agreement of results between these two laboratories ranged from -0.20 for WPI XMRV/P-MLV-positive subjects (no agreement) to 0.21 for all negative controls combined (fair agreement). However, the most telling Kappa value between the WPI and NCI/Ruscetti serology results is the one computed for all subjects combined, which is 0.01 indicating no agreement.

In summary, our study demonstrates that no XMRV/P-MLV assay in any of the nine participating laboratories could reproducibly detect XMRV/P-MLV in fifteen subjects (fourteen with CFS) who had previously been reported as XMRV/P-MLV-infected usually at multiple time points and

often by multiple assays (*2*, *4*). The two laboratories (WPI and NCI/Ruscetti labs) that reported positive results in this study reported similar rates of reactivity among XMRV/P-MLV subjects and known negative control donor samples. The results from both laboratories were inconsistent when their assays were performed in parallel on replicate sample aliquots derived from individual subject specimens. There was also no agreement of reactivity when comparing results between these two laboratories for the 30 blinded XMRV/P-MLV cohorts and control samples. In contrast, assays developed by FDA (Lo and Hewlett), CDC, NCI/DRP, Abbott Diagnostics, Abbott Molecular and Gen-Probe, all of which have been designed to detect XMRV and relevant MLVs with high sensitivity and specificity, failed to detect evidence of viral infection in any of the previously positive subjects, including CFS patients, or negative control specimens represented in the study.

Altogether, 15 XMRV/P-MLV cohort subjects were represented in this study, the maximum number of subjects who could be recruited by the cohort investigators (*2*, *4*). Since most patients were selected based on having previously tested positive for XMRV/P-MLV 1-3 years ago, it is possible that levels of viremia and/or antibody could have waned by the time samples were drawn in our study; however, this is contradictory to Lo et al.'s finding that 4 of 5 patients retested positive 15 years later (*4*). The inconsistent reactive results from the two laboratories that previously reported detection of XMRV (NCI/Ruscetti and WPI) and the negative results from all other laboratories, including the laboratory that previously reported detection of P-MLV (FDA/Lo), strongly suggest that the positive reactivity in this study represents false positive results due to assay non-specificity or cross-reactivity (e.g. to other endogenous or exogenous retroviruses). However, we cannot definitively exclude the possibility that the levels of XMRV/P-MLV markers in blood may be at or below the limit of detection of all assays and/or fluctuate over time as recently described in experimentally infected macaque studies (*22*).

Based on these findings, we conclude that currently available XMRV/P-MLV assays, including the assays employed by the three participating laboratories that previously reported positive results on samples from CFS patients and controls (*2*, *4*), cannot reproducibly detect direct virus markers (RNA, DNA, or culture) or specific antibodies in blood samples from subjects previously characterized as XMRV/P-MLV positive (all but one with a diagnosis of CFS) or healthy blood donors. Finally, our findings are reassuring with respect to blood safety and indicate that routine blood donor screening for XMRV/P-MLV is not warranted at this time.

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Supporting Online Material
www.sciencemag.org/cgi/content/full/science.1213841/DC1
Materials and Methods
SOM Text
Tables S1 to S10
References (*23–35*)
Appendix S1

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Table 1. All XMRV/P-MLV assay results from all laboratories.

Test	Laboratory	Sample type Negative Controls*	WPI XMRV/P-MLV Subjects*	Lo et al. XMRV/P-MLV Subjects*	Spiked Controls*
NAT/Plasma	Abbott-M†	0/15	0/10	0/5	5/5
	CDC	0/15	0/10	0/5	5/5
	FDA/Lo	0/15	0/10	0/5	5/5
	FDA/Hewlett	0/15	0/10	0/5	5/5
	Gen-Probe	0/15	0/10	0/5	5/5
	NCI/DRP	0/15	0/10	0/5	5/5
	WPI	2/15‡	0/10	0/5	3/5
NAT/PBMC	Abbott-M	0/3	0/10	0/5	5/5
	CDC	0/3	0/10	0/5	5/5
	FDA/Lo	0/3	0/10	0/5	5/5
	FDA/Hewlett	0/3	0/10	0/5	5/5
	Gen-Probe	0/3	0/10	0/5	5/5
	NCI/DRP	0/3	0/10	0/5	5/5
	WPI	0/3	1/10‡	0/5	4/5
NAT/WB	Abbott-M	0/15	0/10	0/5	5/5
	CDC	0/15	0/10	0/5	5/5
	FDA/Lo	0/15	0/10	0/5	5/5
	FDA/Hewlett	0/15	0/10	0/5	5/5
	Gen-Probe	0/15	0/10	0/5	5/5
	NCI/DRP	0/15	0/10	0/5	5/5
	WPI	0/15	0/10	0/5	5/5
Culture	FDA/Hewlett	0/15	0/10	0/5	5/5
	NCI/Ruscetti	6/15	3/10‡	0/5	5/5
Serology	Abbott-D	0/15	0/10	0/5	N/A
	CDC	0/15	0/10	0/5	N/A
	NCI/Ruscetti	8/15	3/10	2/5‡	N/A
	WPI	6/15	5/10	5/5‡	N/A

*Number positive/number tested. A single reactive replicate out of 1, 2, or 3 tested for a given individual was considered positive

†Abbott-M, Abbott Molecular; Abbott-D, Abbott Diagnostics; WB, whole blood; N/A, not applicable

‡No significant association was seen when the reactivity rates of control negatives and XMRV/P-MLV cohort subjects were compared [*P* values are discussed (supporting online material text)]

Table 2. Results of replicates for assays with positive results (number reactive/number replicates tested).

Sample Type	Subject	Assay WPI NAT/Plasma	WPI NAT/PBMC	WPI Serology	NCI/Ruscetti Serology	NCI/Ruscetti Culture
Negative Controls						
	1	0/1	0/2	0/2	0/2	0/1
	2	0/1	0/1	0/2	0/2	1/1
	3	0/1	0/1	2/2	1/2	0/1
	4	0/3	NT*	1/3	1/3	0/1
	5	0/3	NT	0/3	0/3	0/1
	6	1/3	NT	0/3	1/3	0/1
	7	0/3	NT	0/3	0/3	0/1
	8	0/3	NT	2/3	0/3	0/1
	9	0/3	NT	3/3	1/3	1/1
	10	0/3	NT	0/3	1/3	1/1
	11	0/3	NT	1/3	1/3	0/1
	12	0/3	NT	1/3	0/3	1/1
	13	0/3	NT	0/3	1/3	1/1
	14	1/3	NT	0/3	1/3	1/1
	15	0/3	NT	0/3	0/3	0/1
				†		
WPI XMRV/P-MLV Subjects						
	1	0/1	0/2	0/2	1/2	0/1
	2	0/1	0/1	1/2	0/2	0/1
	3	0/1	0/1	1/2	0/2	0/1
	4	0/2	0/2	2/2	0/2	1/1
	5	0/1	1/2	0/2	0/2	1/1
	6	0/1	0/2	0/2	0/2	0/1
	7	0/2	0/2	0/2	0/2	0/1
	8	0/1	0/2	1/2	1/2	0/1
	9	0/1	0/3	1/2	0/2	0/1
	10	0/1	0/2	0/2	1/2	1/1
				‡		
Lo et al. XMRV/P-MLV Subjects						
	1	0/3	0/2	1/2	0/2	0/1
	2	0/3	0/2	1/2	0/2	0/1
	3	0/3	0/2	1/2	1/2	0/1
	4	0/3	0/2	1/2	1/2	0/1
	5	0/3	0/1	2/2	0/2	0/1
				§,□		
Spiked Controls						
	1	3/5	4/5	NT	NT	5/5

*NT, not tested.
†The kappa for the serology for the negative controls between NCI/Ruscetti and WPI is 0.21.
‡The kappa for the serology for the WPI XMRV/P-MLV subjects between NCI/Ruscetti and WPI is –0.20.
§The kappa for the serology for the Lo et al. XMRV/P-MLV subjects between NCI/Ruscetti and WPI is 0.00.
□The kappa for the serology for all cohort subjects between NCI/Ruscetti and WPI is –0.08.

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 10. 18	該当なし	

一般的名称	人全血液	研究報告の公表状況	Homaira N, Rahman M, Hossain MJ, Epstein JH, Sultana R, Khan MS, Podder G, Nahar K, Ahmed B, Gurley ES, Daszak P, Lipkin WI, Rollin PE, Comer JA, Ksiazek TG, Luby SP. Epidemiol Infect. 2010 Nov;138(11):1630-6. Epub 2010 Apr 12.	公表国 バングラデシュ
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

○2007年バングラデシュにおけるニパウイルスのヒトーヒト感染の発生
2007年2月、バングラデシュ北西部でニパウイルス(NiV)脳炎のアウトブレイクがあり、7人が感染、そのうち3人が死亡した。一連の患者は、発症中の当該患者と身体的接触後7日～14日後に発症した。症例群をコントロール群と比べると、患者と同室に滞在すること(100%vs.9.5%、P<0.001)や、身体的接触がある(83%vs.0%、P<0.001)方がより発症しやすかった。当該患者の感染原因は明らかになっていないが、アウトブレイク1カ月後の調査で、その地域付近のオオコウモリのうち50%がNiV抗体を持っていたことから、この地域でのウイルスの存在が確認出来る。看病する家族は、潜在的感染源である体液からNiVに感染しないように、予防(石鹸で手を洗う、身体的接触を避ける)を習慣付けることが重要である。

報告企業の意見	今後の対応
バングラデシュにおいて、ニパウイルスのヒトからヒトへの伝播が示唆されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

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Nipah virus outbreak with person-to-person transmission in a district of Bangladesh, 2007

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SUMMARY

In February 2007 an outbreak of Nipah virus (NiV) encephalitis in Thakurgaon District of northwest Bangladesh affected seven people, three of whom died. All subsequent cases developed illness 7–14 days after close physical contact with the index case while he was ill. Cases were more likely than controls to have been in the same room (100% *vs.* 9·5%, OR undefined, $P<0·001$) and to have touched him (83% *vs.* 0%, OR undefined, $P<0·001$). Although the source of infection for the index case was not identified, 50% of *Pteropus* bats sampled from near the outbreak area 1 month after the outbreak had antibodies to NiV confirming the presence of the virus in the area. The outbreak was spread by person-to-person transmission. Risk of NiV infection in family caregivers highlights the need for infection control practices to limit transmission of potentially infectious body secretions.

Key words: Bangladesh, Nipah virus, person-to-person transmission.

INTRODUCTION

In Bangladesh, Nipah virus (NiV) was first identified as the cause of an outbreak of encephalitis in 2001 in Meherpur District [1, 2]. Four additional outbreaks were identified between 2001 and 2005 [1–4]. Antibodies reactive to NiV antigen have been detected in pteropid bats in both India and Bangladesh [1, 5].

Person-to-person transmission of NiV infection, following human infection directly from the environment, was noted in previous outbreaks in the Indian subcontinent. In a NiV outbreak in Siliguri, India in 2001, 45 patients (75%) had a history of hospital exposure to other patients with NiV infection [6]. In Faridpur District, Bangladesh in 2004 NiV case-patients in Faridpur were seven times more likely than

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non-patients to have had close contact with one of the NiV patients [odds ratio (OR) 6·7, 95% confidence interval (CI) 2·9–16·8, $P<0·001$] [2].

On 9 February 2007, a physician at Rangpur Medical College Hospital, one of 10 hospitals involved in active NiV encephalitis surveillance in Bangladesh, reported a cluster of fatal encephalitis involving a husband and a wife residing in the Haripur Upazila (subdistrict) of Thakurgaon District. Both patients had similar symptoms and died within an interval of 2 weeks. A collaborative team including the Institute of Epidemiology Disease Control and Research (IEDCR) and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B), began an investigation on 10 February 2007. The objectives of the investigation were to identify the cause of the outbreak and the risk factors for developing illness.

METHODS

Case definition and identification

We defined suspected case-patients as persons having fever with altered mental status or new onset of seizures (severe illness), or persons having fever with headache or cough (mild illness), residing in the outbreak area with an onset of illness between 15 January and 28 February 2007. The team visited the outbreak village and asked the community health workers and community residents if they were aware of any patient who was suffering from fever with seizure or altered mental status, or who had died from these symptoms in their neighbourhood. We also asked them about case-patients suffering from fever with headache and/or cough. The team then visited the local hospital in order to identify suspected case-patients. Team members also investigated all the deaths in the outbreak village between January and February. We obtained a history of illness and general information about exposures for each suspected case-patient. We asked the local health authority of the affected subdistrict to report to the IEDCR if they identified any further suspected case-patient having fever and altered mental status or seizures who sought treatment in the local subdistrict health complex during February.

The team collected blood samples from living suspected case-patients, which were centrifuged in the field and transported on wet ice to IEDCR, where they were stored at −70°. Samples were tested with an immunoglobulin M (IgM) capture enzyme-linked immunosorbent assay (ELISA) that detects IgM antibodies specific for NiV antigens [7].

We defined a confirmed case of NiV infection as a suspected case-patient with detectable IgM to NiV. The team defined a probable NiV case-patient as a patient with fever and altered mental status who lived in the same village as a confirmed case-patient during the outbreak period, but from whom serum was not available because the patient died before a specimen could be collected.

Qualitative study

A team of experienced anthropologists conducted in-depth interviews and informal discussions with available confirmed and probable case-patients, their family members and friends, and other residents in these communities with the goals of exploring potential exposures to NiV and identifying appropriate proxy respondents for deceased cases or cases that were too sick to interview. The anthropologists also collected information about symptoms of the disease, caregiving practices and health facility utilization by persons affected by the outbreak.

Case-control study

We conducted a case-control study to investigate exposures associated with NiV infection, including person-to-person transmission. Probable and confirmed case-patients were enrolled as cases. We selected three controls for each case-patient. Controls were selected starting from the fourth closest house to the case-patient where no members were ill during the outbreak. The household resident closest in age to the case-patient was eligible to participate as a control. Participation was voluntary. If the selected household resident declined to participate, a resident from the next closest house was asked to participate.

The qualitative team selected proxy respondents for each case-patient who had died or was unable to respond. The proxy respondents included family members and friends of the case-patients who were most knowledgeable about their activities and probable risk exposures in the preceding 1 month before illness. Multiple proxy respondents were common. The investigation team used a standardized questionnaire to collect information on demographics, symptoms of illness, and possible risk factors associated with NiV transmission including history of consumption of date palm juice prior to illness, exposure

to animals and exposure to ill patients, including touching, staying in the same room, feeding, sharing a bed or cleaning body secretions of a NiV patient.

Bat survey

A team of veterinarians from ICDDR,B with assistance from the Consortium for Conservation Medicine located two bat roosts which were 1 km and 15 km distant from the outbreak village. Bats were captured using mist nets and were anaesthetized during sample collection and released at the point of capture after sampling from 24 February to 9 March 2007. All the captured bats from which blood samples were collected were *P. giganteus*.

All bat blood samples were kept on ice until the end of each day when serum was separated and stored in liquid nitrogen. At the end of each day, blood samples were transferred to liquid nitrogen and transported to ICDDR,B where they were stored at −70 °C and then shipped on dry ice to the Australian Animal Health Laboratory for laboratory diagnosis. All the blood samples were assayed for antibodies against NiV using a serum neutralization test.

Statistics

We analysed socio-demographic and clinical profiles of the case-patients using descriptive statistics. For the case-control study, we used ORs to estimate the association of each exposure with disease and calculated 95% CIs around the ORs. We used the χ^2 test when expected cell sizes were >5 and Fisher's exact test when expected cell sizes were <5 and considered association to be statistically significant if the P value was $<0{\cdot}05$. We used an unmatched analysis because neighbours were chosen as controls to ensure that controls and case-patients were representative of the same population and not to control for confounding factors. Because the primary hypothesis was that the index case was the source of NiV transmission for the subsequent cases, we excluded the index case, but none of the controls in the analyses of person-to-person transmission.

Ethics

All human study participants gave informed consent for participation in this investigation. The Ethical Review Committee at ICDDR,B reviewed and approved a protocol for encephalitis surveillance and outbreak investigation. Bat capture and sample collection was conducted under a protocol approved by the Institutional Animal Care and Use Committee.

RESULTS

Descriptive epidemiology

Eleven serum samples were collected from 13 suspected case-patients. Five suspected case-patients had IgM antibodies against NiV by capture ELISA and were thus confirmed cases. Two suspected case-patients had fever and altered mental status, but died before samples could be collected and were categorized as probable cases. These two probable cases were the index case and his wife. The remainder of the analysis was performed on these seven confirmed or probable case-patients. Five of these case-patients (three confirmed and two probable) had fever with altered mental status and three (60%) of them died. A total of five case-patients, including the two probable cases, were hospitalized. The mean age of case-patients was 24 years (range 19–30 years) and five (71%) were male. The median duration from onset of fever to death was 5·6 days (range 5–7) (Table 1). Fever (100%), altered consciousness (71%) along with vomiting (71%) and cough (71%) were the most common symptoms (Table 1).

Qualitative findings

The index case first developed fever on 21 January 2007 which progressed to headache, cough, breathing difficulties, convulsions, loss of consciousness and finally death 5 days later. In total 14 people who were family members, relatives or friends had physical contact with the index case when he was ill; six (43%) of them developed NiV infection. Five of the subsequent cases had contact with the index case only during the last 2 days of his illness (incubation period 7–14 days). The dates of illness onset for subsequent cases ranged from 2 to 8 February 2007 (Fig. 1). None of the caregivers of the subsequent cases developed illness.

During the first 4 days of illness the index case was cared for at home, primarily by his wife and sister-in-law. They fed him, cleaned him and wiped froth and saliva from his mouth. They also massaged oil on his head and body to relieve him of pain. His wife shared the same bed with him and provided care throughout his illness. She became severely ill 14 days

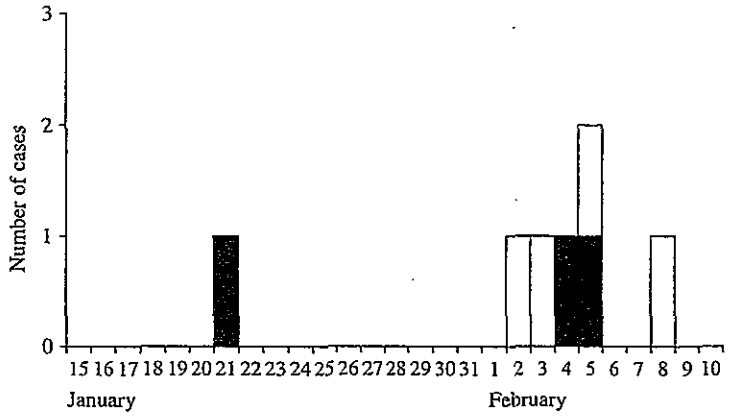

Fig. 1. Distribution of NiV cases by date of onset of illness, Haripur Upazila (subdistrict), Thakurgaon District, Bangladesh, January–February 2007. □, Alive; ■, died.

Table 1. *Characteristics of case-patients, Haripur Upazila, Thakurgaon District, Bangladesh, February 2007*

Characteristics	$n=7$ (%)
Age	
Mean (years)	24
Median (range)	24 (19–30)
Male	5 (71)
Occupation	
Student	2 (29)
Housewife	1 (14)
Businessman	2 (29)
Driver	2 (29)
Clinical feature	
Fever	7 (100)
Severe fatigue/weakness	6 (86)
Headache	3 (43)
Vomiting	5 (71)
Cough	5 (71)
Respiratory distress	4 (57)
Altered mental status	5 (71)
Muscle pain	4 (57)
Restlessness	4 (57)
Unconscious	2 (29)
Joint pain	1 (14)
Case fatality	3 (43)
Onset of illness to death ($n=3$), mean (range)	5·6 (5–7)

after the husband's illness began and died within 6 days of illness.

A day before his death, the index case developed a severe cough and breathing difficulty. He was taken to a local doctor accompanied by a friend and a cousin. A chest radiograph of the index case taken

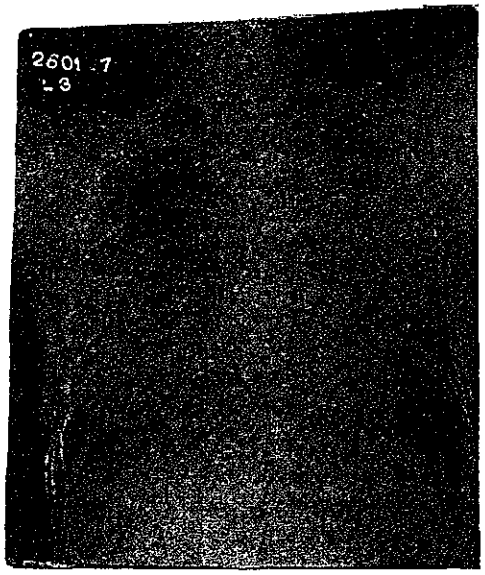

Fig. 2. Chest X-ray of the index case showing features of acute respiratory distress syndrome.

during this period showed diffuse bilateral opacity in both lung fields suggesting features of acute respiratory distress syndrome (Fig. 2). The friend became ill 11 days later and died after 7 days. The cousin also became ill 14 days after his physical contact with the index case.

The day after his chest radiograph, the index case developed reduced level of consciousness, and was admitted to a hospital where he died on the same day. The driver of a micro bus who helped transport and carry him to the hospital developed NiV infection 10 days after exposure.

While the index case was hospitalized, his wife's sister and one of his friends visited him in the hospital

Table 2. *Bivariate analysis of risk factors for Nipah virus infection, Haripur Upazila, Thakurgaon District, Bangladesh, February 2007*

Risk factors	No. (%) of cases with this risk factor	No. (%) of controls with this risk factor	OR	95% CI	*P* value
Male sex	4 (67%)	8 (38%)	3·25	0·48–22	0·2
Climbed trees	1 (17)	4 (19%)	0·85	0·07–9·4	1·00
Physical contact with living animal					
Pig	0 (0%)	0 (%)	Undefined		
Fruit bat	0 (0%)	0 (0%)	Undefined		
Cow	4 (67%)	13 (62%)	1·23	0·182–8·33	1·00
Goat	2 (33%)	9 (43%)	0·67	0·099–4·5	1·00
Ate any animal that had been sick	0 (0%)	1 (5%)	Undefined		1·00
Drank raw date palm sap	1 (17%)	0 (0%)	Undefined		0·22
Visited the index case in a hospital	6 (100%)	0 (0%)	Undefined		<0·001
Touched the index case when he was sick	5 (83·3%)	0 (0%)	Undefined		<0·001
Been present in the same room with the index case when he was sick	6 (100%)	2 (9·5%)	Undefined		<0·001
Been present in the same room with the index case when he was coughing	6 (100%)	0 (0%)	Undefined		0·04

OR, Odds ratio; CI, confidence interval.

and fed and touched him. Both of them developed NiV infection within 10 days of contact.

Case-control study

We used proxy interviews for the three dead case-patients, but not for any controls. The mean age for cases and controls was similar [mean age (±S.D.) 24±4 years in cases *vs.* 24±7 years in controls, $t=-0·097$, $P=0·9$]. Cases were more likely to be males than controls but this could be due to chance (67% males in case-patient group *vs.* 38% in control group, OR 3·2, 95% CI 0·5–22, $P=0·2$).

NiV case-patients were more likely than controls to have consumed raw date palm sap in the 15 days prior to illness (29% in case-patients *vs.* 0% in controls, OR undefined, $P=0·056$). Two (29%) of the case-patients including the index case who had consumed raw date palm juice bought it from a vendor in the local village market. Although there were sick goats in the outbreak-affected community, none of the cases or controls had any contact with them or any other sick animal within 15 days prior to illness. Moreover, there was no report of contact with fruit bats.

In the analysis for person-to-person transmission, case-patients were more likely than controls to have been present in the same room with (100% *vs.* 9·5%, OR undefined, $P=0·000$) or touched (83% *vs.* 0%, OR undefined, $P=0·000$) the index case. In a sub-analysis in those who stayed in the same room with the index case, case-patients were more likely than controls to be present in the same room when he was coughing (100% *vs.* 0%, OR undefined, $P=0·04$). Only case-patients had hospital exposure to other NiV case-patients (86% *vs.* 0%, $P=0·000$), with all reporting visits to the index case in the hospital (Table 2).

Bat study

The team captured and sampled 118 *P. giganteus* bats; 29 of which were juvenile bats. Of the 67 bats sampled 1 km from the outbreak village, 34 (51%) tested positive on serum neutralization test [median titre 30, range 5 to >640]. Three of the 34 serum neutralization test-positive bats had NiV antibody titres >640. In the neighbouring village, 15 km away, 27/51 bats (53%) had serum neutralizing antibodies to NiV [median titre 20, range 5–320]. Of the 61 pteropid bats that were seropositive seven were juvenile bats [median titre 15, range 5–20].

DISCUSSION

Several lines of evidence suggest person-to-person transmission as the primary route of transmission in this outbreak. The epidemic curve showing a gap of

12–18 days between the single primary case and the secondary cases corresponds with the incubation period of human NiV infection [8]. Many of the index case's contacts (43%), who came in physical contact with the index case when he was ill, subsequently became ill. In the case-control study, case-patients were significantly more likely to be in contact with the index case and were significantly more likely to be near him when he was coughing. As subsequent cases were limited to close contacts of the primary case, and none of the contacts of the subsequent cases developed illness, we conclude that the index case was the only NiV transmitter in this outbreak.

Five (83%) of the subsequent cases came in contact with the index case only during the last 2 days of his illness when he developed respiratory symptoms. NiV has been isolated from human saliva, urine, nasal and pharyngeal secretions [9, 10] and there is evidence of spread of NiV infection from direct contact with respiratory secretions or other body secretions of infected pigs and humans [2, 11, 12]. The probability of NiV transmission is probably amplified during the last stages of illness when respiratory symptoms become more prominent and perhaps the concentration of virus in respiratory secretions increases. In Bangladesh, as the level of physical contact with the patient intensifies with the severity of the disease [13], this further increases the risk of transmission.

The NiV neutralizing antibody prevalence was >50% in the bats sampled from the outbreak area which suggests that NiV has circulated in this population of bats. The result is consistent with findings in other pteropid bat populations in Malaysia, India, and Bangladesh [1, 5, 14, 15]. The bat survey was performed approximately 1 month after the onset of illness in the index case, and it is possible that infected bats were present in the colony around the time of the first human infection. Furthermore, the index case had no evidence of exposure to clinically ill domestic animals. He also had history of drinking raw date palm juice before his illness which has been associated with NiV infection in a previous outbreak investigation [3]. These lines of evidence suggest that the virus was probably transmitted directly from its natural reservoir, rather than an intermediate domestic animal.

A limitation of our study is its reliance on proxy interviews for some of the case-patients. This may have obscured some exposure information. However, we started our investigation within 14 days of the death of the index case, and collected information from several proxy respondents thus reducing the likelihood that we failed to collect information on probable exposure to risk factors. Another limitation is the lack of serological data from controls. There is evidence of subclinical infection of NiV from Malaysia [16] which could have reduced power to identify association due to erroneous inclusion of cases as controls. Even with this potential limitation our results identified a biologically credible pathway for transmission.

Findings from outbreaks in Siliguri and Faridpur illustrate that human-to-human transmission has occurred repeatedly in the Indian subcontinent. The social norm in Bangladesh is that family members and loved ones provide hands-on care to sick patients [13]. Further, hospital healthcare workers in Bangladesh are reluctant to provide hands-on care to admitted patients which increases the risk of transmission to family members and relatives who provide care without any training or supplies to reduce the risk of transmission [13]. Efforts to educate caregivers of their risk especially at later stages of illness, while maintaining sensitivity to cultural mores, and promoting basic infection control practices such as washing hands with soap after handling patients and avoiding close physical contact [2] could limit transmission of NiV and other diseases in people who care for sick patients.

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DECLARATION OF INTEREST

None.

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一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	PLoS Pathogens 2011; 7(7)	公表国 アメリカ	
販売名（企業名）	①ヘブスブリン筋注用 200 単位　（ベネシス） ②ヘブスブリン筋注用 1000 単位　（ベネシス） ③ヘブスブリン IH 静注 1000 単位　（ベネシス）					

研究報告の概要

アデノウイルスはヒトとサルを含む多くの脊椎動物に感染し、ヒトで広範囲の臨床疾患を引き起こす。特定株からの感染は、従来、種特異的であると考えられてきた。
カリフォルニア国立霊長類研究所(CNPRC)で、新世界猿(titi サル)の閉鎖集団で集団発生した致命的な新規アデノウイルス(TMAdV:titi サル・アデノウイルス)を特定した。建屋内の 65 匹のサルの間で、23 匹(34%)が劇症の肺炎と肝炎が進行した上気道症状を発症し、うち 19 匹が死亡、または人道的に安楽死させた。
このアデノウイルスは新種で、他のアデノウイルスから高度に分岐し、ヌクレオチド対の同一性は 57%未満であることが TMAdV の全ゲノム配列で分かった。
集団発生の開始時、サルと最も接触した研究者は急性呼吸器疾患を発症し、回復期血清検体は TMAdV により血清陽性であった。また、81 人のランダムな成人献血者のスクリーニングは 2 人(2.5%)に TMAdV-特異の中和抗体を検出した。TMAdV の発見は、新規アデノウイルスが異種間集団発生の潜在的な原因として密接に監視する必要があることを示唆している。

報告企業の意見	今後の対応
アデノウイルスは二重鎖直鎖状DNAウイルスで、カプシドは直径約80nmの正20面体の球形粒子をしており、エンベロープは持たない。万一、原料血漿にアデノウイルスが混入したとしても、EMCおよびCPVをモデルウイルスとしたウイルスクリアランス試験結果から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

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Cross-Species Transmission of a Novel Adenovirus Associated with a Fulminant Pneumonia Outbreak in a New World Monkey Colony

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Abstract

Adenoviruses are DNA viruses that naturally infect many vertebrates, including humans and monkeys, and cause a wide range of clinical illnesses in humans. Infection from individual strains has conventionally been thought to be species-specific. Here, we applied the ViroChip, a pan-viral microarray, to identify a novel adenovirus (TMAdV, titi monkey adenovirus) as the cause of a deadly outbreak in a closed colony of New World monkeys (titi monkeys, *Callicebus cupreus*) at the California National Primate Research Center (CNPRC). Among 65 titi monkeys housed in a building, 23 (34%) developed upper respiratory symptoms that progressed to fulminant pneumonia and hepatitis, and 19 of 23 monkeys, or 83% of those infected, died or were humanely euthanized. Whole-genome sequencing of TMAdV revealed that this adenovirus is a new species and highly divergent, sharing <57% pairwise nucleotide identity with other adenoviruses. Cultivation of TMAdV was successful in a human A549 lung adenocarcinoma cell line, but not in primary or established monkey kidney cells. At the onset of the outbreak, the researcher in closest contact with the monkeys developed an acute respiratory illness, with symptoms persisting for 4 weeks, and had a convalescent serum sample seropositive for TMAdV. A clinically ill family member, despite having no contact with the CNPRC, also tested positive, and screening of a set of 81 random adult blood donors from the Western United States detected TMAdV-specific neutralizing antibodies in 2 individuals (2/81, or 2.5%). These findings raise the possibility of zoonotic infection by TMAdV and human-to-human transmission of the virus in the population. Given the unusually high case fatality rate from the outbreak (83%), it is unlikely that titi monkeys are the native host species for TMAdV, and the natural reservoir of the virus is still unknown. The discovery of TMAdV, a novel adenovirus with the capacity to infect both monkeys and humans, suggests that adenoviruses should be monitored closely as potential causes of cross-species outbreaks.

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Introduction

Adenoviruses, first isolated in the 1950s from explanted adenoid tissue, are double-stranded nonenveloped DNA viruses that naturally infect many vertebrates, including humans and nonhuman primates. The human adenoviruses in the *Mastadenovirus* genus, comprised of all mammalian adenoviruses, are classified into 7 species A-G, and at least 51 different serotypes (and 5 proposed types, HAdV-52 to HAdV-56) have been described to date [1,2]. Adenoviruses are the cause of an estimated 5–10% of febrile illnesses in children worldwide [3]. Some serotypes, such as human adenovirus type 14 (HAdV-14), have been associated with severe and potentially fatal outbreaks of pneumonia in residential facilities and military bases [4]. Adenoviruses have also been associated with other clinical syndromes including conjunctivitis, hepatitis, and diarrhea [5]. In nonhuman primates, most epidemiologic studies of adenoviruses have focused on their identification in fecal samples from asymptomatic animals [6,7,8]. Overt respiratory disease associated with simian adenoviruses has also been observed [9]. Although adenoviruses are significant pathogens, genetically modified strains are being actively explored as potential vectors for vaccines and gene therapy [10].

Infection by adenoviruses has generally been thought to be species-specific. Human adenoviruses do not usually replicate in monkey cells in the absence of helper viruses [11], and do not productively infect rodents (and vice versa) [12]. Studies of sera from animal handlers and zoo workers exposed to chimpanzees in captivity fail to detect antibodies to chimpanzee adenoviruses

Author Summary

Infection from adenoviruses, viruses that cause a variety of illnesses in humans, monkeys, and other animals, has conventionally been thought to be species-specific. We used the Virochip, a microarray designed to detect all viruses, to identify a new species of adenovirus (TMAdV or titi monkey adenovirus) that caused a deadly outbreak in a colony of New World titi monkeys at the California National Primate Research Center (CNPRC), and also infected a human researcher. One-third of the monkeys developed pneumonia and liver inflammation, and 19 of 23 monkeys died or were humanely euthanized. The unusually high death rate (83%) makes titi monkeys unlikely to be natural hosts for TMAdV, and the genomic sequence of TMAdV revealed that it is very different from any other known adenovirus. The researcher developed an acute respiratory illness at the onset of the outbreak, and was found to be infected by TMAdV by subsequent antibody testing. A clinically ill family member with no prior contact with the CNPRC also tested positive. Further investigation is needed to identify whether TMAdV originated from humans, monkeys, or another animal. The discovery of TMAdV suggests that adenoviruses should be monitored closely as potential causes of cross-species outbreaks.

[13,14]. However, recent serological surveys have found antibodies to New World and Old World monkey adenoviruses in donor human sera from regions where the monkeys are endemic [14,15]. In addition, phylogenetic analyses of adenoviruses from greater apes reveal that they fall precisely into "human" adenoviral species B, C, and E [7]. The high degree of sequence relatedness within members of each species suggests that at least some adenoviral strains may be capable of infecting both nonhuman primates and humans.

Beginning in May of 2009, a deadly outbreak of fulminant pneumonia and hepatitis occurred in a closed colony of New World titi monkeys of the *Callicebus* genus at the California National Primate Research Center (CNPRC). Routine microbiological testing for an infectious etiology was negative. We previously developed the Virochip (University of California, San Francisco) as a broad-spectrum surveillance assay for identifying viral causes of unknown acute and chronic illnesses [16,17,18,19,20,21,22]. The Virochip, a pan-viral microarray containing ~19,000 probes derived from all viral species in GenBank (n~2500) [21,23], has been previously successful in detection of novel outbreak viruses such as the SARS coronavirus [22,24] and the 2009 pandemic H1N1 influenza virus [23]. Here we apply the Virochip to identify a novel and highly divergent adenovirus as the cause of the titi monkey outbreak. In addition, we present clinical and serological evidence that this virus may have infected a researcher at the CNPRC and a family member, thus demonstrating for the first time the potential for cross-species infection by adenoviruses.

Results

An outbreak of fulminant pneumonia in a titi monkey colony

In early 2009, the CNPRC housed 65 titi monkeys in one quadrant of an animal building. The index case, a healthy adult titi monkey, presented on May 14, 2009 with cough, lethargy, and decreased appetite (Fig. 1A, T1). Despite aggressive treatment with intravenous fluids and antibiotics, the animal developed severe respiratory distress and was humanely euthanized 5 days later. A second case presented 4 weeks later near the entrance to the building (Fig. 1A, T54). In the interim period, 3 healthy titi monkeys had been relocated from a separate building (Fig. 1A, T2, T3, and T19), with 2 of the 3 monkeys placed into the cage formerly occupied by the index case, reflecting a total at-risk population of 68. Over the ensuing 2 months, 21 additional monkeys, including one of the relocated monkeys, presented with clinical signs similar to those shown by the index case (attack rate = 23/68, or 34%) (Figs. 1A and 1B). Clinical signs in affected animals included cough, lethargy, poor appetite, tachypnea, and abdominal breathing. These symptoms progressed to overt respiratory distress and death or humane euthanasia within an average of 8 days. Chest radiographs typically revealed diffuse interstitial pulmonary changes and bronchoalveolar consolidation indicative of pneumonia, with right middle lobe predominance (Fig. 1C). Animals displaying clinical signs were quarantined and aggressively treated by veterinarians with supplemental oxygen, anti-inflammatory medications, bronchodilators (nebulized albuterol), broad-spectrum antibiotics, and antivirals (oseltamivir and/or ribavirin). In total, 19 animals died or were euthanized due to the illness during the outbreak (case fatality rate = 19/23, or 83%). Only 4 monkeys survived, even though the majority of sick animals (17/23, or 74%) consisted of apparently healthy adults and juveniles. Interestingly, none of the 133 rhesus macaques (*Macaca mulatta*) housed in the same building became sick during the outbreak, and neither did any of the Old World monkeys from surrounding outdoor colonies of rhesus and cynomolgus macaques (*Macaca fascicularis*).

Gross necropsy findings were similar in all titi monkeys and were characterized primarily by diffuse, consolidated pneumonias, with occasional evidence of fibrinous pleuritis, pericardial/pleural edema, and hemorrhage (Fig. 1D-1). Some livers, spleens, and lymph nodes were found to be abnormally enlarged. Hepatic necrosis and hemorrhage, along with ascites, were occasionally appreciated. On histologic examination, the normal cellular architecture of the lung and trachea was destroyed, and prominent intranuclear inclusion bodies were observed in the liver, lung, and trachea (Figs. 1D-2 and 1D-3).

A routine microbiological workup for infectious causes of the outbreak, including bacterial, mycoplasma, and fungal cultures, was negative. Respiratory viral testing failed to detect evidence of respiratory syncytial virus, adenovirus, influenza virus A and B, human metapneumovirus, and parainfluenza virus types 1, 2, and 3.

Virochip identification, PCR screening and electron microscopic (EM) confirmation of TMAdV

Given the clinical presentation of a severe acute viral respiratory illness and the appearance of intranuclear inclusion bodies on histological examination, we strongly suspected that a virus that had eluded detection by conventional assays was the cause of the titi monkey outbreak. Nasal, lung, and liver swab samples collected during necropsy were analyzed using the Virochip [21,23]. Microarrays were analyzed using ranked Z-scores to assess the highest-intensity viral probes [18]. From a lung swab sample from an affected monkey, 4 of the top 80 probes on the Virochip corresponded to adenoviruses. Other viruses or viral families with ≥4 probes among the top 80, including chimpanzee herpesvirus (*Herpesviridae*), bovine viral diarrhea virus (*Flaviviridae*), and endogenous retroviruses (*Retroviridae*), were regarded as less likely to cause fulminant pneumonia and hepatitis, so were not pursued any further. The 4 adenovirus probes mapped to 2 different gene regions corresponding to the DNA polymerase and penton base

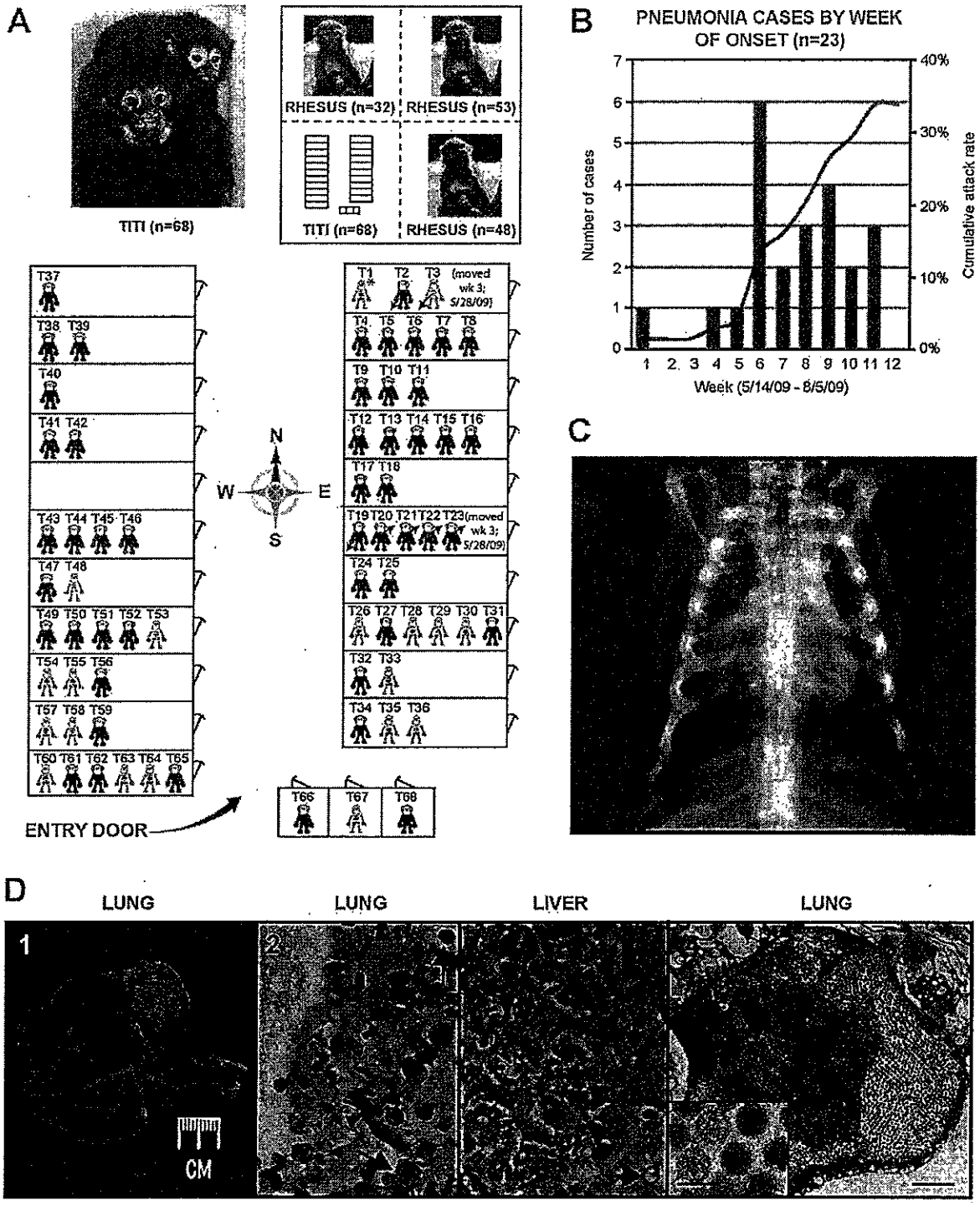

Figure 1. Clinical and epidemiologic features of the titi monkey outbreak. (A) Map of the titi monkey cages situated in one quadrant of a building, showing the locations of asymptomatic, at-risk monkeys (brown or green), affected surviving monkeys (black), and monkeys who died from their illness (skeleton). 3 monkeys were moved into the building (arrows pointing down and to the left) and 4 monkeys out of the building (arrows pointing up and to the right) during the 3rd week of the outbreak. The upper left photograph shows an image of an adult male titi monkey and his infant. The upper right inset shows the location of the titi monkey cages relative to other rhesus monkey cages in the building. Asymptomatic monkeys with positive serum antibody titers to TMAdV 4 months after the outbreak are shown in green. (B) Epidemic curve of the outbreak, with the

number of cases in blue and cumulative attack rate in red. (C) Anteroposterior chest radiograph of an affected titi monkey, showing bilateral basilar infiltrates and a prominent right middle lobe consolidation. (D) *1* – gross photograph of lungs at necropsy; the lungs failed to fully collapse upon opening the chest, and a single ~1.5 cm focus of dark red discoloration (hemorrhage) can be seen in the left caudal lobe. *2* – photomicrograph of H&E stained lung tissue showing a severe diffuse necrotizing bronchopneumonia characterized by the presence of hemorrhage and intranuclear inclusions (arrows). *3* – photomicrograph of H&E stained liver tissue showing a multifocal necrotizing hepatitis with numerous intranuclear inclusions (arrows). *4* – transmission electron micrograph of an affected lung alveolus (scale bar = 1 μm) filled with adenovirus-like particles (inset, scale bar = 0.1 μm).
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(Fig. 2A). Interestingly, the 4 viral probes were derived from 2 different *Adenoviridae* genera (SAdV-23, simian adenovirus 23, PAdV-A, porcine adenovirus A, and HAdV-5, human adenovirus 5, in the *Mastadenovirus* genus; FAdV-D, fowl adenovirus D, in the *Aviadenovirus* genus), suggesting the presence of a divergent adenovirus that was not a member of any previously known species.

To confirm the Virochip finding of an adenovirus, we used consensus primers to amplify a 301 bp fragment from the hexon gene by PCR [25]. The fragment shared ~86% nucleotide identity with its closest phylogenetic relatives in GenBank, SAdV-18, an Old World vervet monkey adenovirus, and the human species D adenoviruses. The newly identified adenovirus was designated TMAdV, or titi monkey adenovirus. Specific PCR for TMAdV was then used to screen body fluids and tissues from affected monkeys (Table 1). PCR results were positive from post-necropsy liver and lung tissues as well as from sera, conjunctival swabs, oral swabs, and nasal swabs collected at time of quarantine in 8 different affected monkeys, but were negative from a throat swab from an asymptomatic animal whose other 5 cage mates had become sick. In addition, nasal swabs were negative in 3 asymptomatic, minimal-risk titi monkeys housed in a separate building. To confirm the presence of virus in diseased tissues, we examined lung tissue from affected monkeys by transmission electron microscopy, revealing abundant icosahedral particles characteristic of adenovirus filling the alveoli (Fig. 1D-4).

Next, to assess persistent subclinical infection from TMAdV, we analyzed serum samples from at-risk asymptomatic or affected surviving monkeys 2 months after the outbreak (n = 41). All post-outbreak serum samples were negative for TMAdV by PCR (Table 1). To assess potential TMAdV shedding, stool samples collected from all cages housing titi monkeys 2 months post-outbreak were analyzed by PCR (n = 27), and were found to be negative. In addition, we checked for TMAdV in rectal swab samples from rhesus macaques housed in the same building as the titi monkeys (n = 26) and in pooled droppings from wild rodents (n = 2) living near the titi monkey cages. All macaque and rodent fecal samples were negative for TMAdV by PCR.

We also sought to determine whether PCR assays commonly used to detect human adenoviruses in clinical or public health settings could detect TMAdV. Adenovirus PCR was performed on a TMAdV-positive clinical sample, a TMAdV culture, and a human adenovirus B culture (as a positive control) using an additional 5 pairs of primers, according to previously published protocols [26,27,28]. Three of the 5 primer pairs, designed to detect human respiratory adenoviruses B, C, and E, failed to amplify TMAdV [27]. The remaining 2 pairs of primers, both derived from highly conserved sequences in the hexon gene [26,28], were able to detect TMAdV in culture as well as directly from clinical material.

Whole-genome sequencing, features, and phylogenetic analysis of TMAdV

To facilitate whole-genome sequencing of TMAdV, deep sequencing of a lung swab from one affected titi monkey and lung tissue from another affected monkey was performed. Out of ~11.9 million high-quality reads, 2,782 reads and 3,767 reads aligned to the SAdV-18 genome by BLASTN (Fig. 2B, blue) and TBLASTX (Fig. 2B, transparent blue), respectively, with reads mapping to sites across the genome. *De novo* assembly of the complete TMAdV genome from reads that aligned to SAdV-18 was not possible due to insufficient sequence coverage (<46%). The poor apparent coverage was the result of high sequence divergence of the TMAdV genome from SAdV-18, which hindered the identification of most of the 16,524 actual deep sequencing reads derived from TMAdV (Fig. 2B, red). Thus, after partial assembly of TMAdV using overlapping reads aligning to the SAdV-18 genome, remaining gaps were closed by specific PCR. The complete genome of TMAdV was found to be 36,842 base pairs in length, with a base composition of 20.8% A, 29.8% C, 29.8% G, and 19.6% T, and a GC content of 59.6%, comparable to that of adenoviral species Groups C, D, and E in the *Mastadenovirus* genus. The deduced genomic structure of TMAdV was also similar to that of other mastadenoviruses and consists of 34 open reading frames (Fig. 2C).

Whole-genome phylogenetic analysis placed TMAdV in an independent species group separate from the known human adenoviral species A–G (Fig. 3). Among all 95 fully-sequenced adenovirus genomes in GenBank, the closest simian adenoviral relatives to TMAdV were SAdV-3, SAdV-18, and SAdV-21, with pairwise nucleotide identities ranging from 54.0% to 56.3% (Fig. 4). The closest human adenoviral relatives were the species D adenoviruses, which share 54.3% to 55.1% identity to TMAdV, with human adenoviruses of other species slightly less similar (51.1%–54.6%). The placement of TMAdV into a separate group by phylogenetic analysis was also observed when looking individually at the hexon, polymerase, penton base, and fiber genes (Fig. S1). Scanning nucleotide pairwise identity plots revealed that, among the major adenovirus genes, the DNA polymerase and hexon are more conserved, whereas the E1A and fiber are more divergent (Fig. 4). The significant overall sequence divergence of TMAdV from known human and simian adenoviruses is highlighted by the finding that PAdV-A (porcine adenovirus A), a non- primate mammalian adenovirus, shared only a slightly less similar whole-genome pairwise identity to TMAdV of 47.0% (Fig. 4). In fact, in the DNA polymerase gene, TMAdV shared a pairwise identity with PAdV-A of 67.2%, comparable to its pairwise identities with the other human adenoviruses, 59%–71.7% (Figs. 4 and S1). Although TMAdV was found to be highly divergent from other adenoviruses, different isolates of TMAdV from 3 affected titi monkeys were remarkably conserved, sharing 100% identity across the full-length hexon gene (data not shown).

The high level of sequence divergence in TMAdV held true at the amino acid level as well, with amino acid identities relative to other mastadenoviruses ranging from 20.8% to 27.5% for the fiber, the most divergent protein, to 68.7%–78.2% for the hexon (Table 2). Although bearing low sequence similarity to other adenoviruses, the penton base of TMAdV contained an RGD motif that presumably binds α_v integrins. By both nucleotide and amino acid comparisons, the closest phylogenetic relative to

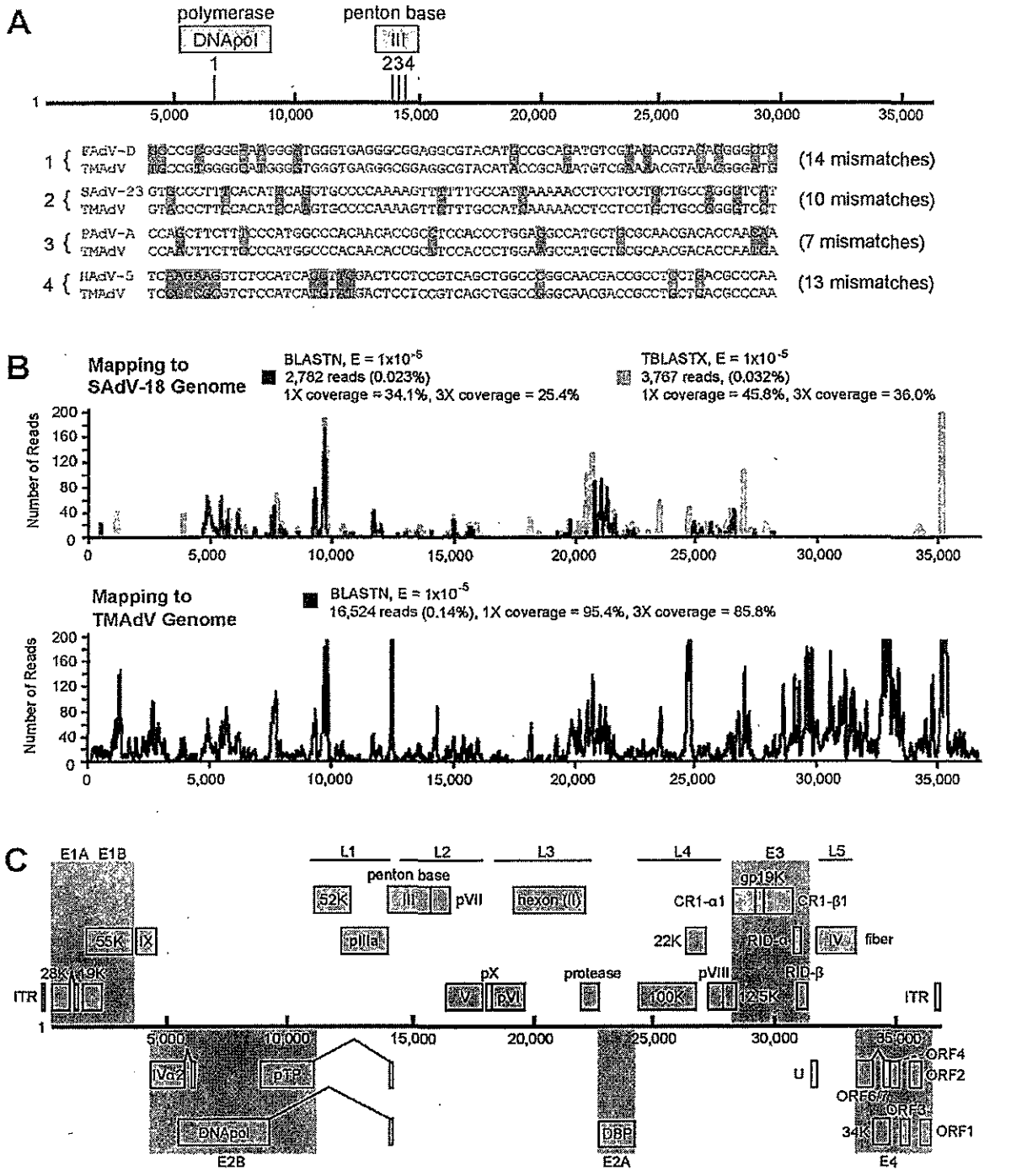

Figure 2. Discovery and whole-genome characterization of the novel adenovirus TMAdV. **(A)** The locations of the 4 Virochip probes derived from adenovirus sequences and used to detect TMAdV are mapped onto the ~37 k8 genome. The 4 Virochip probe sequences are also aligned with the corresponding sequence in the TMAdV genome, with mismatches highlighted in pink. **(B)** Coverage map of deep sequencing reads corresponding to TMAdV using BLASTN (blue) and TBLASTX (transparent blue) alignments to SAdV-18. The actual coverage achieved by deep sequencing as determined by alignments to the fully sequenced genome of TMAdV is much higher (red). **(C)** Genome organization of TMAdV. Predicted protein coding regions are shown as boxes. Boxes above the central black line represent open reading frames (ORFs) that are encoded on the forward strand, while boxes underneath the black line represent reverse-strand encoded ORFs. Early region ORFs are shaded in gray. The x-axis refers to the nucleotide position along the ~37k genome of TMAdV. Abbreviations: FAdV, fowl adenovirus; SAdV, simian adenovirus; PAdV, porcine adenovirus; HAdV, human adenovirus, TMAdV, titi monkey adenovirus.
doi:10.1371/journal.ppat.1002155.g002

Table 1. PCR screening for TMAdV.

Sample	Sample Type	PCR Result	Date Presenting with Clinical Signs	Date of Necropsy
Affected, at-risk titi monkeys (died)				
T1	serum[$]	–	5/14/2009	5/19/2009
T26	serum[¶]	+	7/23/2009	7/30/2009
T28	conjunctival swab[¶]	+	7/16/2009	7/25/2009
	nasal swab[¶]	+		
	liver swab[¶]	+		
	lung swab[¶]	+		
T29	serum[¶]	+	7/26/2009	7/31/2009
T30	serum[¶]	–	7/25/2009	7/30/2009
T33	lung swab[¶]	+	6/23/2009	6/29/2009
	nasal swab[¶]	+		
T36	lung swab[¶]	+	7/7/2009	7/14/2009
	lung swab[¶]	+		
T60	serum[¶]	–	7/15/2009	7/22/2009
T63	serum[¶]	–	6/20/2009	8/1/2009
T67	nasal swab[#]	–	7/7/2009	8/13/2009
	nasal swab[¶]	+		
Affected, at-risk titi monkeys (survived)				
T31	serum[*]	–	7/10/2009	N/A
T32	serum[*]	–	7/12/2009	N/A
T34	serum[*]	–	6/23/2009	N/A
T62	serum[*]	–	7/8/2009	N/A
Asymptomatic at-risk and minimal-risk titi monkeys				
T27	throat swab (n = 1)[¶]	–	N/A	N/A
at-risk titi	stool from cages (n = 14)[*]	–	N/A	N/A
at-risk titi	serum (n = 29)[*]	–	N/A	N/A
minimal-risk titi	oral swab (n = 3)[¶]	–	N/A	N/A
minimal-risk titi	stool from cages (n = 5)[*]	–	N/A	N/A
minimal-risk titi	serum (n = 9)[*]	–	N/A	N/A
minimal-risk titi	stool from cages (n = 8)[*]	–	N/A	N/A
minimal-risk titi	serum (n = 8)[*]	–	N/A	N/A
Other				
rhesus	rectal swabs (n = 26)[*]	–	N/A	N/A
human	serum (n = 15)[∞]	–	N/A	N/A
rodent	droppings (n = 2)[*]	–	N/A	N/A

For titi monkey cage designations (TXX), please refer to Fig. 1.
[$]initial case.
[#]collected prior to outbreak.
[¶]collected during outbreak.
[*]collected 2 months after outbreak.
[∞]collected 4 months after outbreak.
doi:10.1371/journal.ppat.1002155.t001

TMAdV in GenBank overall was SAdV-3 (Fig. 4; Table 2). Bootscanning analysis revealed no evidence for recombination of TMAdV with other adenoviruses at either the whole-genome or individual gene level (Fig. S2).

The main neutralization determinant for adenoviruses, the epsilon determinant (ε), is formed by loops 1 and 2 in the hexon protein [29]. The epsilon determinant of TMAdV was significantly divergent from that of other mastadenoviruses, with amino acid identities in loop 1 varying from 30.6% to 44.8% and in loop 2 varying from 54.4% to 67.0% (Table 2). This observation suggested that cross-neutralization of TMAdV with sera reactive against other human/simian adenoviruses is unlikely.

Cultivation of TMAdV in human and monkey cells

We next attempted to culture TMAdV in an A549 (human lung adenocarcinoma) cell line, a BSC-1 (African green monkey kidney epithelial) cell line, and PMK (primary rhesus monkey kidney) cells (Fig. 5). Direct inoculation of cell cultures with a lung swab sample from an affected titi monkey produced a weak initial cytopathic

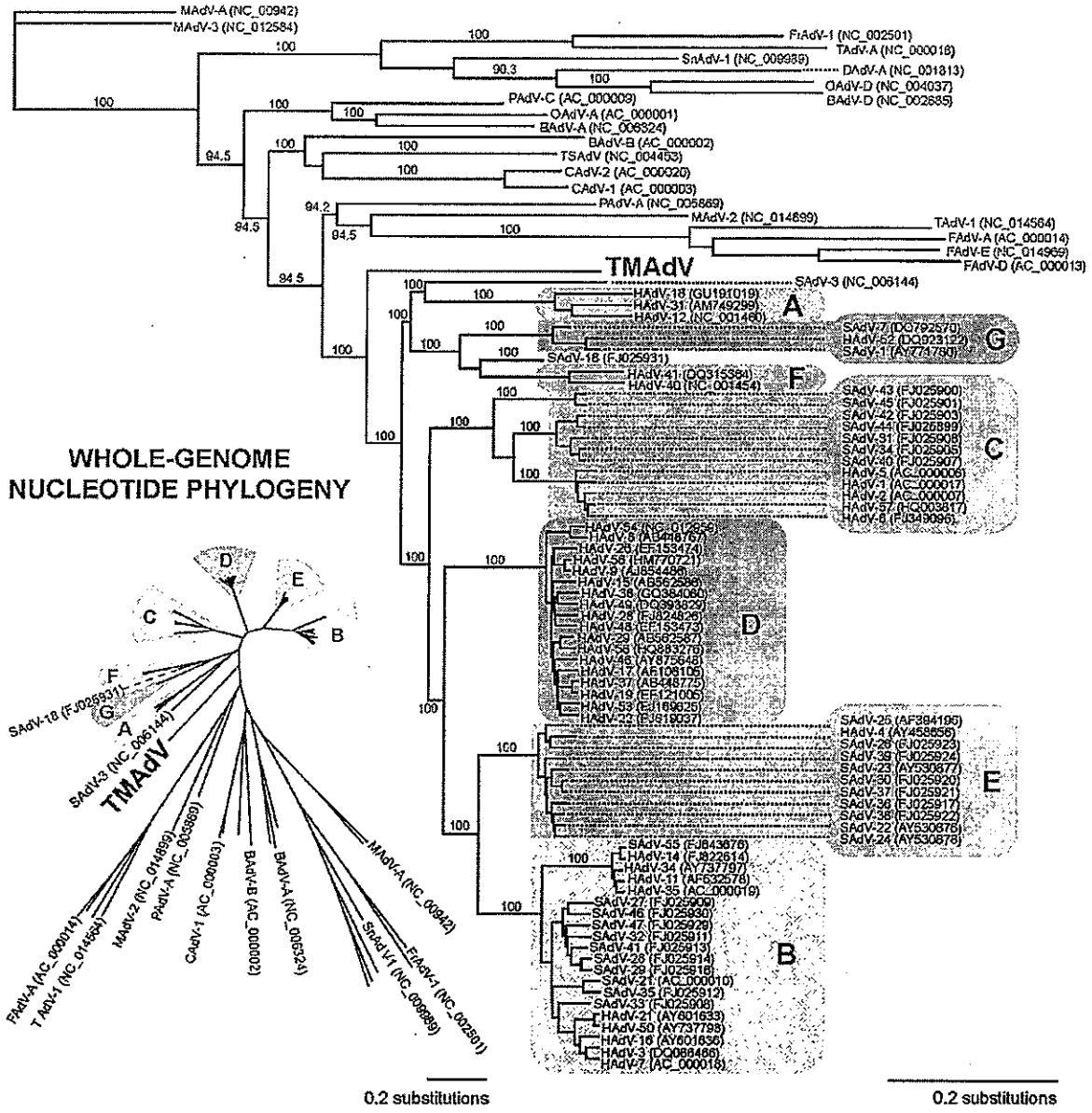

Figure 3. Whole-genome phylogenetic analysis of TMAdV. The whole-genome nucleotide phylogenetic tree is reconstructed from a multiple sequence alignment of all 95 unique, fully-sequenced adenovirus genomes in GenBank and TMAdV. Both rectangular cladogram and radial tree layouts are displayed. The branch corresponding to TMAdV is highlighted in boldface red. Abbreviations: HAdV, human adenovirus; SAdV, simian adenovirus; MAdV, mouse adenovirus, FrAdV, frog adenovirus; TAdV, turkey adenovirus; SnAdV, snake adenovirus; DAdV, duck adenovirus; OAdV, ovine adenovirus; BAdV, bovine adenovirus; PAdV, porcine adenovirus; TSAdV, tree shrew adenovirus; CAdV, canine adenovirus.
doi:10.1371/journal.ppat.1002155.g003

effect in macaque BSC-1 and human A549 cells at day 7. However, despite multiple serial passages, we were unable to propagate the infected cell culture supernatant in either BSC-1 or PMK cells. In contrast, propagation in human A549 cells resulted in viral adaptation by passage 6 and generation of a fully adapted strain of TMAdV by passage 10 that was able to productively infect all 3 cell lines. Thus, culturing and propagation of TMAdV were successful in a human A549 cell line, but not in established or primary monkey kidney cell lines.

An influenza-like illness in a researcher and family members during the titi monkey outbreak

In hindsight, only one individual at the CNPRC reported becoming ill during the titi monkey outbreak, the researcher in closest, daily contact with the animals. Symptoms began near the onset of the outbreak, although whether they began prior to or after identification of the index case is unclear. The researcher, with a past medical history of multiple sclerosis, initially developed symptoms of a viral upper respiratory infection (URI), including

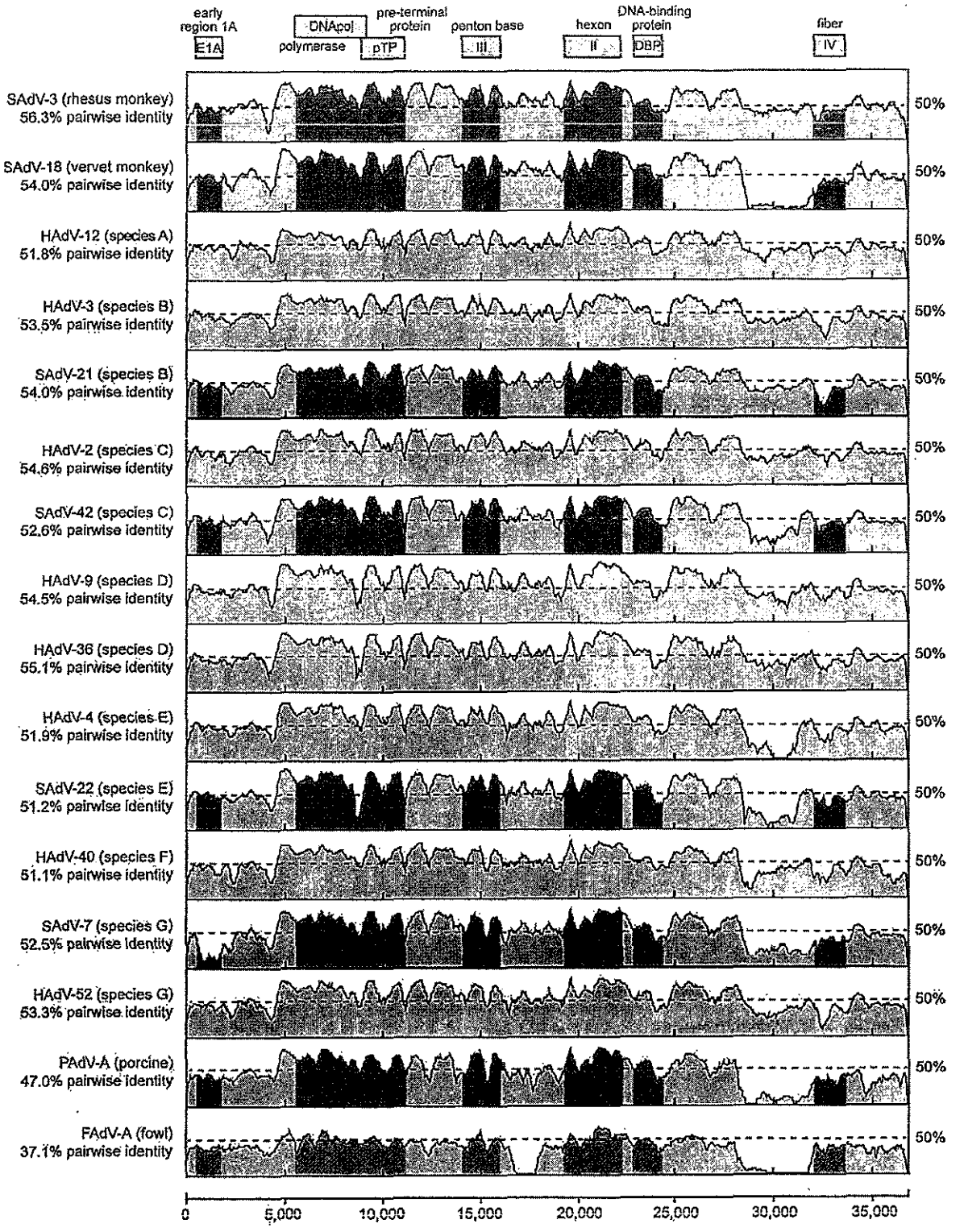

Figure 4. Scanning pairwise alignment of representative adenoviruses with TMAdV. The scanning nucleotide pairwise identities of TMAdV relative to representative human (yellow) or simian (brown) adenoviruses in species A–G, porcine adenovirus (red), and fowl adenovirus (green) are shown. The window size is 400 bp with a step size of 40 bp. The x-axis refers to the nucleotide position along the ~37 k genome of TMAdV. Abbreviations: HAdV, human adenovirus; SAdV, simian adenovirus; PAdV, porcine adenovirus; FAdV, fowl adenovirus.
doi:10.1371/journal.ppat.1002155.g004

Table 2. Amino acid identity of TMAdV relative to other adenoviruses.

	fiber (IV)	E1A	DBP	DNApol	penton base (III)	pTP	hexon (II) whole	hexon (II) ε:L1	hexon (II) ε:L2
SAdV-3 (rhesus monkey)	26.6%	29.9%	37.9%	61.8%	68.2%*	70.6%*	78.2%*	44.8%*	67.0%*
SAdV-18 (vervet monkey)	26.0%	30.7%	39.1%	63.2%	66.5%	68.3%	76.7%	39.1%	63.9%
HAdV-12 (species A)	26.4%	31.0%	38.2%	60.4%	64.3%	67.6%	76.2%	40.1%	64.9%
HAdV-3 (species B)	22.3%	31.4%	36.5%	61.7%	65.8%	68.8%	73.7%	38.3%	63.9%
SAdV-21 (species B)	22.2%	30.6%	36.0%	62.1%	66.1%	68.8%	72.2%	34.1%	61.2%
HAdV-2 (species C)	25.0%	32.6%*	39.5%	62.5%	67.0%	68.0%	71.4%	39.1%	61.4%
SAdV-42 (species C)	26.4%	30.5%	38.8%	62.5%	66.5%	68.1%	72.4%	37.4%	63.3%
HAdV-9 (species D)	21.9%	28.4%	38.3%	63.4%	66.1%	68.3%	74.0%	33.3%	60.0%
HAdV-36 (species D)	20.8%	30.0%	38.1%	63.3%	65.9%	68.3%	73.6%	33.1%	61.9%
HAdV-4 (species E)	26.3%	32.6%*	37.1%	62.8%	67.9%	69.8%	72.7%	30.6%	63.3%
SAdV-22 (species E)	27.5%*	31.2%	36.8%	62.9%	67.6%	70.4%	74.2%	38.6%	63.9%
HAdV-40 (species F)	26.9%	31.0%	40.1%*	62.1%	64.1%	65.9%	77.1%	42.6%	66.3%
SAdV-7 (species G)	25.5%	32.6%*	34.9%	63.3%	67.0%	68.0%	76.4%	43.8%	59.6%
HAdV-52 (species G)	24.1%	30.3%	35.6%	63.8%*	67.8%	67.9%	77.0%	46.2%*	62.6%
PAdV-A (porcine)	26.4%	23.6%	37.4%	55.4%	61.7%	57.7%	68.7%	36.4%	54.4%
FAdV-A (fowl)	1.6%	N/A	25.8%	36.5%	41.6%	31.7%	47.9%	22.3%	39.0%

The amino acid sequences of selected TMAdV proteins and the epsilon determinant of the hexon (ε: L1, loop 1, and ε: L2, loop 2) are compared to the corresponding proteins from representative human, simian, porcine, and fowl adenoviruses.

*For each protein, the entry corresponding to the adenoviral species with the highest percentage identity relative to TMAdV.

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fever, chills, headache, and sore throat, followed by a dry cough and "burning sensation in the lungs" that was exacerbated by a deep breath or coughing. The researcher endorsed a history of recurrent upper respiratory infections, and did not regard the illness as related to the titi monkey outbreak. Although symptoms persisted for 4 weeks, at no time did the researcher seek medical care, and no antibiotics were taken during the illness.

We carried out contact tracing to identify family members and other individuals in close contact with the researcher. Interestingly, two family members in the household also developed flu-like symptoms about 1–2 weeks after the researcher initially became sick. Their symptoms – fever, cough and muscle aches – appeared milder than those of the researcher and completely resolved within 2 weeks. Neither individual sought medical care for these symptoms, and notably, neither had ever visited the CNPRC.

Seroprevalence of TMAdV in monkeys and humans

To explore a potential link between the outbreak and associated illness in humans, we blindly tested available sera from titi monkeys (n = 59), rhesus macaques housed in the same building (n = 36), CNPRC personnel and close contacts (n = 20), and random human blood donors (n = 81) for evidence of recent or prior infection by TMAdV by virus neutralization (Fig. 6). Nineteen serum samples from 15 at-risk affected (symptomatic) titi monkeys were tested. Among 3 affected titi monkeys surviving the outbreak, 2 monkeys mounted a vigorous neutralizing Ab response to TMAdV, with negative pre-outbreak Ab titers (<1:8) but antibody titers 2 months after the outbreak of >1:512, while 1 monkey exhibited a positive but much weaker response. Affected titi monkeys who died during the outbreak exhibited a wide range of neutralizing Ab titers, from <1:8 to >1:512 (those without Ab likely died before mounting a response).

To investigate the possibility of subclinical infection by TMAdV, we also examined serum samples from asymptomatic titi monkeys (n = 40) and nearby rhesus macaques (n = 36), collected 2 months after the outbreak. Fourteen of 40 asymptomatic titi monkeys tested (35%) had antibody to TMAdV, indicating that the incidence of subclinical infection was significant (Fig. 1A; Fig 6). In fact, one of the 14 asymptomatic titi monkeys with positive Ab titers was located in the minimal-risk building. In contrast, only 1 of 36 rhesus macaque samples was positive, with an Ab titer of 1:16. The 1 antibody-positive rhesus serum sample was negative by specific PCR for TMAdV (data not shown), as was stool from the cage in which the rhesus monkey was housed (Table 1).

Approximately 4 months after the outbreak, serum samples were collected from CNPRC personnel in direct contact with the titi monkeys. Serum samples were also collected from the two family members of the clinically ill CNPRC researcher 1 year after the outbreak. Only two samples were found positive for neutralizing Abs to TMAdV: (1) Ab titers for the clinically ill researcher were 1:32, and (2) Ab titers for one of the family members of the clinically ill researcher were 1:8.

Among 81 random blood donors from the Western United States, 2 individuals (2/81, 2.5%) had positive Ab titers of 1:16 and 1:8. Pooled rabbit sera containing antibodies to human adenovirus serotypes 1 through 35, representing species A–E, were unable to neutralize TMAdV (data not shown). Thus, the results of our serological survey appear unlikely to be due to nonspecific cross-reactivity from prior exposure to known human adenoviruses.

Discussion

In this study, we employed a pan-viral microarray assay, the Virochip, to identify a novel adenovirus associated with a fulminant pneumonia outbreak in a colony of New World titi monkeys. Despite the absence of an animal model, which precludes a strict fulfillment of Koch's postulates, there are several lines of evidence implicating this novel adenovirus, TMAdV, as

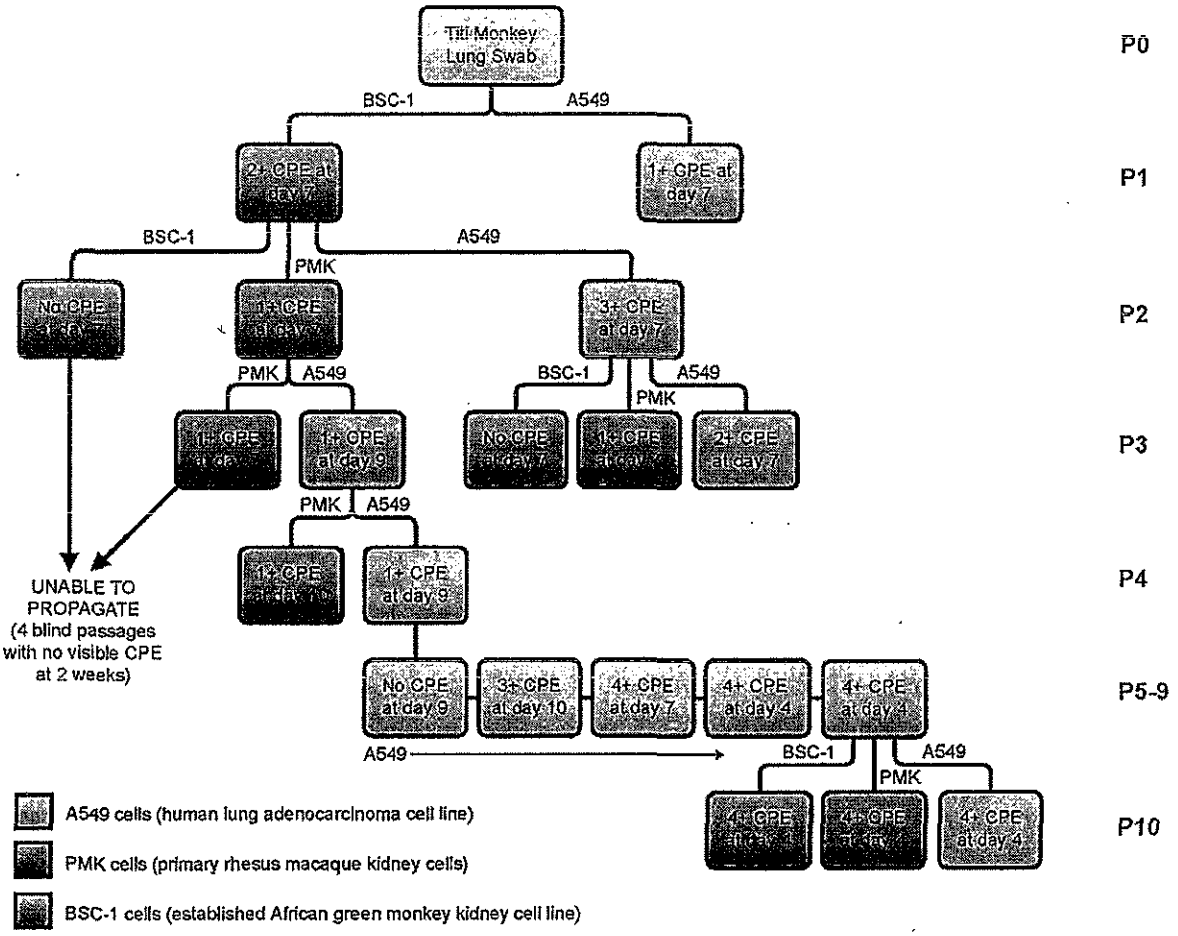

Figure 5. Growth and propagation of TMAdV in cell culture. The flow chart displays 10 passages (P1–P10) of TMAdV cultured in human lung adenocarcinoma (A549, orange), primary rhesus macaque kidney (PMK, brown), or established African green monkey kidney (BSC-1, green) cells.
doi:10.1371/journal.ppat.1002155.g005

the cause of the outbreak. First, conventional testing for other pathogens, including other viruses by Virochip, was negative, and affected monkeys did not respond to empiric therapy with antibiotics or antivirals (ribavirin and oseltamivir in anecdotal use are not effective against adenoviral infections) [30]. Second, the clinical presentation of pneumonia and hepatitis is consistent with the known spectrum of disease associated with adenoviral infections. Third, TMAdV sequence was recovered by PCR in various body fluids and tissues from affected monkeys, including blood, respiratory secretions, and lung/liver tissue (Table 1). Fourth, the finding of intranuclear inclusions in diseased tissues, as well as direct visualization of adenoviral-like particles (TMAdV) in lung alveoli by electron microscopy (Figs. 1D-2 to 1D-4), support a primary role for TMAdV in the pathogenesis of tissue injury in affected monkeys. Finally, there was a significant neutralizing Ab response in surviving animals, with 2 monkeys having titers undetectable prior to the outbreak but rising to >1:512 at convalescence (Fig. 6).

Although TMAdV retains the core genomic features common to all adenoviruses (Fig. 2C), phylogenetic analysis clearly places TMAdV within a separate branch, with no closely related neighbors (Figs. 3 and S1). A phylogenetic distance of >10% combined with the lack of cross-neutralization defines TMAdV as a new species [31]. Since emerging adenovirus strains such as HAdV-14 and HAdV-D22/H8 (otherwise known as HAdV-D53) are known to arise from recombination events among related ancestral strains [32,33], we performed bootscanning analysis to look for such events in TMAdV. The bootscanning analysis, however, failed to show evidence of recombination, likely because closely related and/or ancestral strains to TMAdV have not yet been identified.

Entry of adenoviruses into cells involves an initial attachment of the fiber knob to the cell receptor, followed by internalization via a secondary interaction of the penton base with α_v integrins [34,35]. The presence of an RGD motif in the TMAdV penton base implies that the virus uses α_v integrins for internalization [35]. However, the high sequence divergence in the fiber protein (Table 2), as well as the absence of fiber motifs conserved among adenoviruses that bind CAR [36,37] (coxsackievirus-adenovirus receptor) or CD46 [38,39,40] (data not shown), suggest that neither of these two human adenoviral receptors may be the attachment receptor for TMAdV. Further studies will be necessary to identify the preferred cellular attachment and internalization receptors for TMAdV.

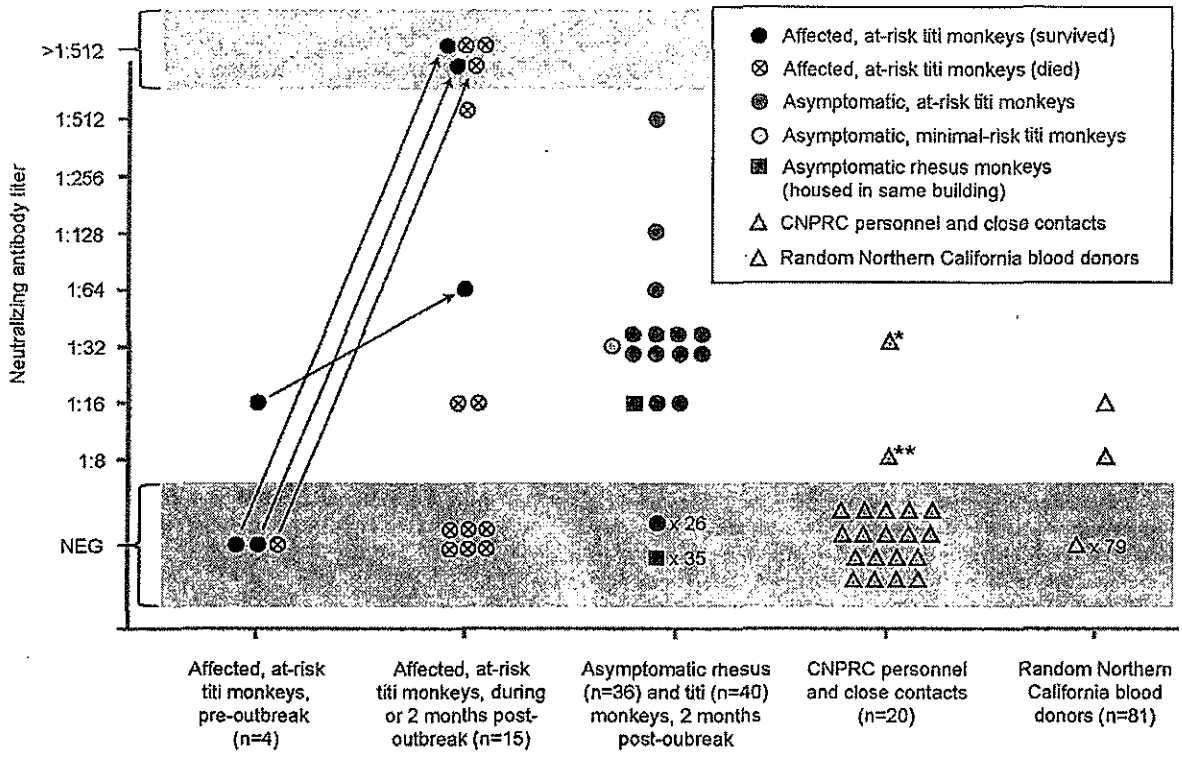

Figure 6. Seroprevalence of TMAdV in humans and monkeys. Sera from titi monkeys (circles), rhesus macaques (squares), and humans (triangles) were tested for antibodies to TMAdV by virus neutralization. Arrows designate pre-outbreak and post-outbreak serum samples from the same individual monkey. Pre-outbreak serum samples were previously banked in 2007. Sera from CNPRC personnel and close contacts (orange triangles) were collected 4 months post-outbreak, except for the two family members of the clinically ill researcher, whose sera were collected 1 year post-outbreak. *, clinically ill researcher; **, family member of the researcher, who was also sick. Abbreviations: CNPRC, California National Primate Research Center; NEG, negative.
doi:10.1371/journal.ppat.1002155.g006

Despite its isolation from affected titi monkeys, we were unable to propagate TMAdV in both established (BSC-1) and primary (PMK) monkey kidney cells (Fig. 4). The virus, however, grew efficiently in a human A549 lung adenocarcinoma cell line. One explanation for this finding is that TMAdV may be unable to productively infect cells derived from Old World monkeys (e.g. rhesus and African green monkeys). An alternative possibility is that successful propagation of TMAdV may depend on infection of a specific host cell type, such as A549 lung, and not BSC-1 or PMK kidney cells. Nevertheless, after 10 passages in human A549 cells, the fully adapted strain of TMAdV exhibits an extended host range with the ability to productively infect both monkey and human cells. This observation implies that TMAdV possesses an inherent capacity to cross the species barrier and infect both humans and nonhuman primates. Efforts to identify host range and cell tropism of TMAdV, as well as the specific sequence changes responsible for adaptation to growth in cell culture, are currently underway.

The virulence of TMAdV in healthy and apparently immunocompetent titi monkeys (83% case fatality rate) is highly unusual for infections by adenovirus. In humans, deaths due to adenovirus infections or outbreaks are generally low (up to 18% for pneumonia associated with HAdV-14 [4]). Furthermore, severe infections from human adenoviruses are more commonly associated with older age, immunosuppression, and chronic underlying conditions such as kidney failure [4,41]. Young, healthy individuals are in general much less likely to succumb to adenoviral-related illness. The severity of TMAdV-related illness in affected titi monkeys suggests that this species of monkey may not be the natural host for the virus. The failure to detect fecal shedding of TMAdV in convalescent or asymptomatic animals also suggests that the virus does not normally infect titi monkeys (Table 1).

Although the exact origin of TMAdV remains unclear, we can speculate on several possibilities. One possibility is that a cross-species "jump" from captive macaques to a susceptible colony of titi monkeys precipitated the outbreak. As there have been no new introductions of monkeys into the closed colony for the past 2 years, this conjecture relies on asymptomatic infection and transmission of TMAdV in the captive rhesus/cynomolgus macaque population at the CNPRC. CNPRC personnel who visited macaque areas would occasionally enter titi rooms with no change in personal protective equipment, thus providing a potential route of transmission for the virus. In addition, specific antibodies were detected in 1 of 36 (2.8%) asymptomatic rhesus macaques housed in the same building (Fig. 6), indicating that TMAdV has the capacity to infect this species of Old World monkey. Notably, the closest identified phylogenetic relative to TMAdV among the complete genomic sequences available in GenBank is a rhesus monkey adenovirus, SAdV-3 (Fig. 4; Table 2). Furthermore, serological evidence for cross-species adenoviral transmission events between different nonhuman primate species has been previously reported in the literature [42].

Although we failed to detect TMAdV in rodent droppings found near titi monkey cages (Table 2), it is still possible that the virus arose from an unknown animal reservoir. In this regard, the high sequence divergence of TMAdV relative to the known human/simian adenoviruses (Fig. 3), and comparable sequence similarity in the polymerase gene to a porcine adenovirus (Figs. 3 and S1) are striking. The four-week interval between the index case and the second case appears overly long given a typical incubation period for adenovirus infections of no more than 1 week [43]. This may be explained by our finding of a high rate of subclinical infection by TMAdV in asymptomatic titi monkeys (35%), but may also be due to separate introductions of TMAdV into the colony from an as-yet unidentified reservoir.

Our study data also support the potential for cross-species transmission of TMAdV between monkeys and humans. The researcher's fever, cough, and pleuritic symptoms ("burning sensation in the lungs") are consistent with the development of a prolonged viral respiratory illness. Interestingly, pleurisy has been specifically reported in association with certain human adenovirus infections [44]. The clinical presentation, time of illness concurrent with the onset of the outbreak, and presence of neutralizing Abs in convalescent serum all strongly point to primary infection of the researcher by TMAdV. The detection of weakly neutralizing Abs (1:8) in a serum sample from a sick family member of the researcher also suggests that TMAdV may be capable of human-to-human transmission. The decreased levels of neutralizing Abs to TMAdV in the researcher (1:32) and a family member (1:8) relative to those in infected titi monkeys (up to >1:512) are consistent with a recent study showing much higher levels of neutralizing antibodies in chimpanzees than in humans with adenovirus infections, possibly due to more robust adenovirus-specific T-cell responses in humans than in monkeys [45].

Several lines of evidence support the contention that the direction of TMAdV transmission was zoonotic (monkeys to humans) rather than anthroponotic (humans to monkeys). First, the closest known relative to TMAdV in GenBank is SAdV-3, an Old World monkey adenovirus (Fig. 3; Table 2). Second, our results show that PCR assays for human adenoviruses in common use are capable of detecting TMAdV. Although sequencing of PCR amplicons for human adenoviruses is not performed routinely in diagnostic virology, TMAdV would presumably have been detected previously in large-scale studies of hexon sequencing of Ad field isolates if it were circulating in the community [46,47]. Finally, the available sequence data in GenBank is heavily biased towards human adenoviruses, and much less is known about the potential diversity of the simian adenoviruses. We also cannot formally exclude the possibility that the outbreak arose from anthroponotic transmission. In our study, 2 of 81, or 2.5% of random adult blood donors exhibited borderline titers of neutralizing antibody to TMAdV, indicating either a low prevalence of TMAdV in the human population or cross-reactivity to a related virus (although no evidence of cross-reactivity was found with HAdV serotypes 1 through 35). Future large-scale studies of TMAdV seroepidemiology will be needed to better understand transmission of TMAdV between monkeys and humans. Nevertheless, our discovery of TMAdV, a novel adenovirus with the capacity to cross species barriers, highlights the need to monitor adenoviruses closely for outbreak or even pandemic potential.

Materials and Methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The use and care of all animals followed policies and guidelines established by the University of California, Davis Institutional Animal Care and Use Committee (IACUC) and CNPRC (Animal Welfare Assurance #A3433-01). The protocol for the maintenance and breeding of the titi monkey colony was approved by the University of California, Davis IACUC (Protocol #15730). No specific animal research protocol was drafted for this study, as only excess clinical samples were analyzed for diagnostic purposes. Animals in extreme respiratory distress were humanely euthanized by veterinarians. Extensive veterinary care was provided to all animals affected by the outbreak in order to minimize pain and distress.

Serum samples from staff at the CNPRC, close contacts, and random adult blood donors were collected under protocols approved by institutional review boards of the University of California, Davis (Protocol #200917650-1) and University of California, San Francisco (Protocol #H49187-35245-01). Specifically, written informed consent was obtained from staff at the CNPRC and close contacts for analysis of their samples. Any potentially identifying information has been provided with the explicit permission of the individuals involved.

Sera from random blood donors were obtained from the Blood Systems Research Institute (San Francisco, CA); sera were derived from affiliated donor banks in California (Blood Centers of the Pacific, San Francisco, CA), Nevada (United Blood Service, Reno, NV), and Wyoming (United Blood Services, Cheyenne, Wyoming) and de-identified prior to analysis.

The California National Primate Research Center (CNPRC)

The California National Primate Research Center (CNPRC), which houses over 5,000 nonhuman primates, is a part of the National Primate Research Centers Program and is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALAC). At the beginning of 2009, the CNPRC maintained a colony of 74 titi monkeys (*Callicebus cupreus*) and a colony of over 4,500 rhesus macaques (*Macaca mulatta*). No new animals have been introduced into either colony for over 2 years. All titi monkeys are maintained in small social groups, while rhesus macaques are maintained in small or large social groups. All animal facilities are maintained in compliance with United States Department of Agriculture specifications.

Eighty-eight percent of the titi monkey population (n = 65) were housed in 1 quadrant of an indoor animal building, and all titi monkeys demonstrating clinical signs originated from this building (i.e. "at-risk" room) (Fig. 1A). Rhesus macaques (n = 133) were housed in the other 3 quadrants of this same building, and surrounding the building were outdoor housing units with rhesus macaques and cynomolgus macaques (*Macaca fasicularis*). Three additional titi monkeys were moved into the at-risk room less than 2 weeks after presentation of the index case, reflecting a total at-risk population of 68 animals. The remaining 6 titi monkeys were housed in an indoor animal building greater than 500 yards from the at-risk population (i.e. "minimal-risk" room).

Outbreak investigation and microbiological testing

The outbreak lasted approximately 3 months from May to August of 2009. Affected titi monkeys died from 3–24 days after appearance of clinical signs, with an average time to death or humane euthanasia of 8 days. Clinical and epidemiological data, including daily census reports, were tracked and recorded by veterinary and management staff. All personnel entering the titi monkey rooms (both at-risk rooms and minimal-risk rooms)

needed to pass within approximately 20 feet of macaque enclosures prior to entry. CNPRC personal protective equipment (PPE) policy requires a change of PPE between entrance/exit of animal rooms housing different species. Staff compliance of this policy may have been compromised. Measures have since been taken by CNPRC management to ensure compliance with existing policies.

Bacterial, mycoplasma, and fungal cultures were performed at the CNPRC. Clinical samples were also sent to an outside laboratory (Focus Diagnostics, Cypress, CA) for respiratory viral testing by centrifugation-enhanced shell vial culture followed by direct fluorescent antibody staining for 8 viruses (respiratory syncytial virus, adenovirus, influenza virus A and B, parainfluenza virus types 1, 2, and 3, and human metapneumovirus).

Gross, histopathological, and ultrastructural analyses

Gross and histopathological analyses of post-mortem tissues were performed by a board-certified veterinary pathologist specializing in nonhuman primate/laboratory animal medicine, a branch of Primate Services at the CNPRC. At necropsy, tissue samples from the trachea, lung, and liver were collected and fixed in 10% formalin. Tissues were routinely processed and embedded in paraffin. 3-µm sections were stained with hematoxylin and eosin (HE) and examined by light microscopy. For transmission electron microscopy, tissue fragments (2×2 mm) were excised from paraffin blocks of lung, deparaffinized, and processed as previously described [48].

Nucleic acid extraction and cDNA library preparation

Total nucleic acid was extracted from body fluid or swab samples using commercially available kits (Qiagen, Valencia, CA). 200 µL of sample were passed through a 0.22 µm filter (Millipore, Temecula, CA) to remove bacteria and cellular debris and then treated with Turbo DNase (Ambion, Culver City, CA) to degrade host genomic DNA prior to extraction. For tissue samples, lung or liver tissue was homogenized in a 15 mL Eppendorf tube using a disposable microtube pestle (Eppendorf, San Diego, CA) and scalpel, and RNA extraction was then performed using TRIzol LS (Invitrogen, Carlsbad, CA), followed by isopropanol precipitation and two washes in 70% ethanol. Extracted nucleic acid was amplified using a random PCR method to generate cDNA libraries for Virochip and deep sequencing analyses as previously described [18,21].

Virochip analysis

The current 8×60 k Virochip microarrays used in this study contain 19,058 70mer probes representing all viral species in GenBank, and combine probes from all previous Virochip designs [17,18,21,23]. Four probes derived from 2 different *Adenoviridae* genera (SAdV-23, PAdV-A, HAdV-5, and FAdV-D) yielded an adenovirus signature on the Virochip that was found to be TMAdV. With the exception of SAdV-23, these highly conserved probes are part of the core Virochip design and were derived from all available adenoviral sequences in GenBank as of 2002 [21]. One explanation why more high-intensity probes to simian adenoviruses were not seen by Virochip analysis is that the genomes of many simian Ads, including SAdV-3 and SAdV-18 (the two closest phylogenetic relatives to TMAdV in GenBank), were not sequenced until after 2004 [7,49], and thus their genomes are not as well-represented on the Virochip microarray.

Virochip analysis was performed as previously described [21,23]. Briefly, samples were labeled with Cy3 or Cy5 fluorescent dye, normalized to 10 pmol of incorporated dye, and hybridized overnight using the Agilent Gene Expression Hybridization kit (Agilent Technologies, Santa Clara, California). Slides were scanned at 3 µm resolution using an Agilent DNA Microarray Scanner. Virochip microarrays were analyzed with Z-score analysis [18], hierarchical cluster analysis [50], and E-Predict, an automated computational algorithm for viral species prediction from microarrays [51]. Only Z-score analysis, a method for assessing the statistical significance of individual Virochip probes, yielded a credible viral signature on the microarray.

PCR screening

We initially used consensus primers derived from a highly conserved region of the hexon gene to confirm the Virochip finding of an adenovirus by PCR [25]. After recovering the full genome sequence, we then designed a set of specific PCR primers for detection of TMAdV, TMAdV-F (5′-GTGACGTCA-TAGTTGTGGTC) and TMAdV-R (5′-CTTCGAAGGCAAC-TACGC). The TMAdV-specific quantitative real-time PCR was performed on a Stratagene MX3005P real-time PCR system using a 25 µL master mix consisting of 18 µL of water, 2.5 µL of 10X *Taq* buffer, 1 µL of $MgCl_2$ (50 mM), 0.5 µL of deoxynucleoside triophosphates (dNTPs; 12.5 mM), 0.5 µL of each primer (10 µM), 0.5 µL SYBR green, 0.5 µL of *Taq* polymerase (Invitrogen, Carlsbad, CA), and 1 µL of extracted nucleic acid. Conditions for the PCR reaction were 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. Amplicons were purified on a 2% agarose gel, cloned into plasmid vectors using TOPO TA (Invitrogen, Carlsbad, CA), and sent to an outside company (Elim Biopharmaceuticals, Hayward, CA) for Sanger sequencing in both directions using vector primers M13F and M13R.

To assess linearity and limits of sensitivity for the TMAdV PCR assay, 12 serial log dilutions were made of a standard plasmid constructed by cloning the 157-bp TMAdV amplicon into a TOPO plasmid vector. Purified plasmid clones at each serial dilution were quantified using a Nanodrop spectrophotometer and then spiked into negative serum, stool, or oral swab sample matrix, each matrix consisting of a pool of 10 sera, 10 stool samples, or 3 oral swabs, respectively. For each sample type, a standard curve for the TMAdV PCR assay was calculated from 3 PCR replicates at each dilution of nucleic acid extracted from the spiked matrix (data not shown). To determine limits of sensitivity for the assay, probit analysis of results from 6 PCR replicates of 7 serial log dilutions (from a starting concentration of $\sim 1.2\times10^5$ copies/mL) was performed using SPSS 16.0 (SPSS Inc., Chicago, IL). By probit analysis, the 95% limit of detection for TMAdV was 781, 377, or 35 viral genome equivalents/mL for serum, stool, or oral swab samples, respectively (data not shown).

Whole-genome sequencing

To facilitate whole-genome sequencing of TMAdV, we prepared amplified cDNA/DNA libraries for deep sequencing from lung tissue and a lung swab sample from 2 different monkeys using previously published protocols [23,52]. Briefly, randomly amplified libraries were cleaved with a Type IIs restriction endonuclease (*GsuI*) and truncated adapters were ligated on the resulting strand ends. Full-length adapters containing strict 6-nt barcodes were added via an additional 15 cycles of PCR. Amplified libraries were size-selected on a 2% agarose gel at approximately 350 bp average length and then sent to an outside company (Elim Biopharmaceuticals, Hayward, CA) for deep sequencing on an Illumina Genome Analyzer IIx (Illumina, San Diego, CA). Paired-end reads were sequenced for 73 cycles in each direction. Paired-end reads were subsequently filtered to eliminate low-complexity sequences with a Lempel-Ziv-Welch (LZW) compression ratio below 0.4 [53], split into individual reads,

classified by barcode, and stripped of any remaining primer sequences using BLASTN alignments (word size = 11, E-value = 1×10^{-5}). After low-complexity filtering and barcode/primer trimming, 11,950,557 sequence reads remained, with each read consisting of 67 nucleotides, for a total of ~800 megabases of sequence. Reads were then aligned using BLASTN (word size = 11, E-value = 1×10^{-5}) and TBLASTX (word size = 11, E-value = 1×10^{-5}) to the genome sequence of SAdV-18 (Fig. 2B). Overlapping reads aligning to SAdV-18 were used to assemble portions of the TMAdV genome with Geneious software (version 3.6.5) [54], employing the SAdV-18 genome as a reference sequence and requiring a 20-bp minimum overlap and 95% overlap identity. Aligning reads were also used to design PCR primers to close remaining gaps in the TMAdV genome. Amplicons derived from specific TMAdV PCR primers were gel-purified, cloned, and sequenced as described above. The 5′ end corresponding to the inverted terminal repeat (ITR) of TMAdV was obtained by PCR using a forward degenerate consensus primer and a reverse TMAdV-specific primer. The 3′ end was recovered using a forward primer near the 3′ end of the genome and a reverse primer derived from 5′-ITR sequence.

Structural features and phylogenetic analysis

To identify predicted coding regions in the TMAdV genome, we used the fully annotated genome sequence of SAdV-21 in GenBank as a reference. First, we aligned the two genomes and identified all ORFs that were present with Geneious [54]. We then selected the candidate ORF that best matched the corresponding ORF in the annotated reference genome. For adenovirus genes that are spliced (e.g. E1A), the identification of a GT-AG intron start-stop signal was used to pinpoint the correct ORF. To confirm the accuracy of the coding sequence, the sequence of each identified ORF was aligned to a database containing all adenoviral proteins in GenBank by BLASTX.

To generate whole-genome and individual gene nucleotide phylogeny trees, all 95 fully sequenced unique adenovirus genomes were first downloaded from GenBank. Multiple sequence alignments were then performed on a 48-core Linux system using ClustalW-MPI [55]. Trees were constructed after bootstrapping to 1000 replicates by the neighbor-joining method (based on Jukes-Cantor distances) in Geneious [54,56]. Pairwise alignments were calculated using Shuffle-LAGAN (window size, 400 bp; step size 40 bp; translated anchoring), a glocal alignment algorithm that is able to calculate optimal alignments by using both local alignments and global maps of sequence rearrangements (e.g. duplications of the fiber gene in adenovirus genomes with 2 fibers) [57]. Sliding window analysis of the Shuffle-LAGAN pairwise alignments was performed using the online mVISTA platform [58]. More accurate alignments were obtained with Shuffle-LAGAN than with either ClustalW-MPI or Geneious (data not shown). Bootscanning analysis was performed according to the Kimura 2-parameter method using 1000 replicates with Simplot (version 3.5.1) [59]. Pairwise amino acid amino acid alignments between predicted TMAdV proteins and corresponding proteins in other adenoviruses (Table 2) were performed using Geneious [54].

Virus cultivation

A549 (human lung adenocarcinoma) and BSC-1 (African green monkey kidney epithelial) cell lines as well as PMK (primary rhesus monkey kidney) cells are routinely maintained at the Viral and Rickettsial Disease Laboratory (VRDL) branch of the California Department of Public Health. Media consisting of Hank's medium (for A549 cells) or Dulbecco's modified Eagle's medium (DMEM) (for BSC-1 cells) were supplemented with 1×nonessential amino acids (Invitrogen, Carlsbad, CA), 10% fetal bovine serum, 100 U of penicillin/mL and 100 µg of streptomycin/mL. PMK cells were maintained in tubes containing growth media and antibodies to SV-40 and SV-5 polyomaviruses (Viromed, Pasadena, CA). Clinical samples were clarified by centrifugation for 10 min×4000 *g* and passaged through a 0.2-µm filter. Cell culture passages were subjected to 3 freeze-thaw cycles and clarified as above. After achieving 80–90% confluency, cell culture media were changed to maintenance media with 2% FBS and were inoculated with 200 µL of clinical sample or 100 µL of passaged viral supernatant. Viral replication was monitored over 14 days by visual inspection under light microscopy for cytopathic effect (CPE). To confirm the generation of infectious virus, viral supernatants were quantitated by an end-point dilution assay.

Virus neutralization assay (human and monkey sera)

A virus stock of TMAdV (passage 7) was produced on human A549 cells, aliquoted, and quantitated by end-point dilution. To perform the virus neutralization assay, 55 µL of viral supernatant at a concentration of 100 $TCID_{50}$ and 55 µL of serum (starting at a 1:8 dilution) were mixed and incubated for 1 hour at 37°C. As a control for each serum sample, 55 µL of culture media and 55 µL of serum were mixed and treated in an identical fashion. While mixtures were incubating, A549 cells grown in T-25 plates were trypsinized and 4,000 cells in 100 µL of media were added to each well of a 96-well plate. After incubation, 100 µL of mixture were inoculated into appropriate wells containing 4,000 cells per well and the entire plate was placed in a 37°C 5% CO_2 incubator. Cells in the plate wells were observed for evidence of CPE every other day for 1 week. For wells that showed inhibition of viral CPE, the corresponding serum samples were diluted in six 2-fold steps and then retested. The reciprocal of the highest dilution that completely inhibited viral CPE was taken as the neutralizing antibody titer.

Virus neutralization assay (rabbit typing sera)

To assess cross-neutralization of TMAdV by known human adenoviruses, 7 pools of in-house reference sera at the VRDL (rabbit hyperimmune sera) and collectively containing antibodies to human adenovirus serotypes 1 through 35 were available for testing. For each pool, 55 µL of rabbit sera and 55 µL of viral supernatant at a concentration of 100 $TCID_{50}$ were mixed, incubated for 1 hour at 37°C, and inoculated onto A549 cells in wells of a 96-well plate as described above. Cells in the plate wells were observed for evidence of CPE every other day for 1 week.

Microarray and nucleotide sequence accession numbers

All Virochip microarrays used in this study have been submitted to the NCBI GEO database (study accession number GSE26898; microarray accession numbers GSM662370-GSM662391; microarray design accession number GPL11662). The annotated, whole-genome sequence of TMAdV has been submitted to GenBank (accession number HQ913600). Deep sequencing reads have been submitted to the NCBI Sequence Read Archive (accession number SRA031285).

Supporting Information

Figure S1 Phylogenetic analysis of the hexon, polymerase, penton base, and fiber genes of TMAdV. A multiple sequence alignment of selected genes from all 95 unique, fully-sequenced adenovirus genomes in GenBank and TMAdV is performed and the results displayed as a radial phylogenetic tree. The branch corresponding to TMAdV is highlighted in boldface

red. Abbreviations: HAdV, human adenovirus, SAdV, simian adenovirus; PAdV, porcine adenovirus; FAdV, fowl adenovirus.
(TIF)

Figure S2 Bootscanning recombination analysis of TMAdV. Bootscanning analysis was initially performed with all 95 unique, fully-sequenced adenovirus genomes in GenBank (data not shown). After removal of similar viral genomes, bootscan plots of the whole genome and individual genes from a subset representing human/simian adenoviruses in species A–G and all non-primate vertebrate adenoviruses were generated. The window size is 400 bp with a step size of 40 bp for the whole genome, and 200 bp with a step size of 20 bp for the individual genes. The x-axis refers to the nucleotide position. For definition of abbreviations, please refer to Fig. 3.
(TIF)

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Author Contributions

Conceived and designed the experiments: ECC SY KLB DPS NWL CYC. Performed the experiments: ECC SY SPM NM AR AS CYC. Analyzed the data: ECC SY KRK NM KLB DPS NWL CYC. Contributed reagents/materials/analysis tools: ECC SY SPM NM AR AS KLB DPS NWL CYC. Wrote the paper: ECC SY KRK KLB DPS NWL CYC.

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別紙 3-1

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一般的名称			研究報告の公表状況	http://www.phac-aspc.gc.ca/ccdrw-rmtch/2011/ccdrw-rmtcs2911-eng.php#b	公表国 カナダ	
販売名（企業名）						

研究報告の概要	使用上の注意記載状況・その他参考事項等
2009 年後半、カリフォルニアデービスにある米国立霊長類研究センターの titi monkey に病原体不明の感染症が流行し、飼育されていた 65 匹中 23 匹のサルが感染して重症肺炎になり 19 匹が死亡した（死亡率 83%）。死亡したサルの肺組織から新種のアデノウイルスが見つかり TMAdV（titi monkey adenovirus）と名付けられた。 この感染事故発生初期にこれらのサルと濃厚接触していた研究者が、発熱、悪寒、頭痛、咳、肺の灼熱感といった、アデノウイルス感染が疑われる症状を呈した。また、研究者の家族 2 人も軽度だが似た症状が認められた。この 3 人は治癒し、ウイルスそのものを検出することはできなかったが、血中に TMAdV に対する抗体が検出されたため、このウイルスに感染していたと考えられた。アデノウイルスは種特異性があり、サルのアデノウイルス、ヒトのアデノウイルス等その動物種のみで感染することが示されており、サルからヒトへの感染（あるいはその逆）はこれまで知られていなかったが、今回、異種間で同一のアデノウイルス感染が認められた。また、軽度の症状を発現した研究者の家族は感染サルへの接触がないことから、このウイルスがヒトからヒトに感染した可能性が示唆された。一方、このウイルスがヒトからサルに伝染した可能性も、完全には、否定できないと報告されている。	BYL-2011-0407 PLoS Pathogenens Vol.7 No.7 ; e1002155; 1-16, 2011

報告企業の意見	今後の対応
今回、アデノウイルス科の新たなウイルス種が同定され、アデノウイルスでは初めて動物種を越えた感染（人畜共通感染）が認められた。また、この新型アデノウイルスである TMAdV はヒトーヒト感染も起こすことが確認された。 TMAdV の本来の宿主は不明であるが、本報告にあるような霊長類施設以外で人が感染する確率は低いと考えられる。また、TMAdV に感染したヒトの症状は、人アデノウイルスによるそれと類似していると報告されている。このことから、現時点で、TMAdV がヒトに重大な脅威となるとは考えにくい。本剤の精製工程は、エンベロープを有さないウイルスに対し十分な不活性化なし除去能を有することが示されている。TMAdV に類するウイルスの混入の可能性はきわめて低いと考えられる。 しかしながら、アデノウイルスは種特異性を有するという定説が覆されたことから、新たな人畜共通感染について注視していく必要がある。	現時点で新たな安全対策上の措置を講じる必要はないと考える。 今後も，新規人畜共通感染症や新たなウイルス感染症に関する情報収集に努める。

Canada Communicable Disease Report
CCDR Weekly

Infectious Diseases News Brief - July 22, 2011

[Current Issue - Table of contents]

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Archived Issues
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Surveillance
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West Nile Virus

Measles And Water-Borne Disease Outbreaks In Horn Of Africa And Kenya Worries World Health Organization

The numbers of people becoming infected with measles and water-borne diseases is growing at an alarming rate in the Horn of Africa and some neighboring countries, says WHO (World Health Organization). Cases of severe diarrhea in Kenya and Ethiopia are a serious concern, the organization adds. Severe drought in the Horn of Africa and Kenya is making millions of people move to other areas, an important factor in the spread of communicable diseases. WHO predicts the problem will get worse. The Horn of Africa, also known as Northeast Africa or the Somali Peninsula is in northern east Africa, it is a peninsula that sticks out hundreds of miles into the Arabian sea. The Horn of Africa has approximately 100 million people, and includes Ethiopia, Somalia, Eritrea and Djibouti. The affected countries have low immunization rates, a shortage of clean water, poor sanitation and extremely poor health care systems.

Source: Medical News Today 17 July 2011
http://www.medicalnewstoday.com/articles/231242.php

First Adenovirus to Jump Between Monkeys and Humans Confirmed

A novel virus that spread through a California monkey colony in late 2009 also infected a human researcher and a family member, UCSF researchers have found, the first known example of an adenovirus "jumping" from one species to another and remaining contagious after the jump. In a study by the UCSF Viral Diagnostics and Discovery Center, which identified the new virus at the time of the outbreak, researchers confirmed it was the same virus in the New World titi monkeys and the two humans. They also confirmed that the virus is highly unusual in both populations, suggesting that it may have originated from a third, unidentified species. The direction in which the virus spread, however -- from monkeys to humans or vice versa -- remains a mystery. Findings appear in the July 14 issue of *PLoS Pathogens*, a weekly journal of the Public Library of Science. The virus, which researchers have named titi monkey adenovirus (TMAdV), infected more than a third of the titi monkeys in the California National Primate Research Center (CNPRC) in late 2009. In the monkeys, the virus was devastating, causing an upper respiratory illness that progressed to pneumonia and eventually killed 19 of the 23 monkeys (83 percent) that became sick, including healthy young adult monkeys. Around the time of the

outbreak, a researcher who was taking care of the sick monkeys also developed an upper respiratory infection, with fever, chills and a cough that lasted four weeks, as did two members of the researchers' family who had no contact with the monkey colony. All three recovered fully without medical treatment. The primate center called Chiu when the illness spread through the colony to help identify the pathogen and prevent its spread to other animals. The UCSF Viral Diagnostics and Discovery Center specializes in using a microarray Virochip technology developed at UCSF to identify viruses affecting humans, animals, insects or plants.

Because the researcher's illness was not reported for several months, the virus could no longer be detected directly, so Chiu worked with the California Department of Public Health to conduct antibody testing on the monkeys, the researcher and two of the researcher's family members who also reported having been sick. Antibodies are a product of the body's immune response to pathogens and generally remain in the bloodstream for several months after infection. As a result, they serve as a marker of whether a person was exposed to a specific virus. Both the monkeys and researcher tested positive for antibodies to the TMAdV virus, as did one of the two family members. No other humans at the center were found to have been infected. The UCSF team found that the new virus clearly belonged to the adenovirus family, yet was unlike any adenovirus ever reported to infect humans or monkeys, including from large-scale studies by public health agencies such as the U.S. Centers for Disease Control and Prevention. The new virus was so unusual, in fact, that it shares only 56 percent of its DNA to its closest viral relative.

Source: Science Daily News July 15 2011
http://www.sciencedaily.com/releases/2011/07/110714191427.htm

Date Modified: 2011-07-22

Top of Page

Important Notices

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	EID Vol.17 No.8; Available from: http://wwwnc.cdc.gov/eid/article/17/8/11-0285_article.htm	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○米国でのヒトにおける新種のアレナウイルス感染
アレナウイルスに属する、ホワイトウォーターアロヨウイルス(WWAV)と他7種類から成るノースアメリカンタカリベセロコンプレックスウイルス(NATSV)及びリンパ球性脈絡髄膜炎ウイルス(LCMV)は、北米で発生することが知られている。5種類のサウスアメリカンタカリベセロコンプレックスウイルス(SATSV)、LCMV、ラッサ熱ウイルスはヒトにおける深刻な発熱性疾患の病原体であるが、NATSVのヒトへの影響は正確に調査されていない。
米国で急性中枢神経疾患や原因不明の熱性疾患の患者1,185人中41人(3.5%)から抗WWAV/抗LCMV-IgG抗体が検出された。ペア血清サンプルの抗体価の分析結果から、NATSVが2人、LCMVが3人の疾患原因であると示唆された。この研究結果は、NATSVもLCMVと同様に米国内でヒトの疾患原因となることを示している。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、
細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
今までヒトへの影響が正確に調査されていなかったノースアメリカンタカリベセロコンプレックスウイルスに対するIgG抗体が米国の急性中枢神経疾患や原因不明の熱性疾患の患者から検出され、ヒト疾患の原因となることが示唆されたとの報告である。	日本赤十字社では、輸血感染症対策として受付時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、発熱などの体調不良者を献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

15

Novel Arenavirus Infection in Humans, United States

Mary Louise Milazzo, Grant L. Campbell, and Charles F. Fulhorst

Immunoglobulin G against Whitewater Arroyo virus or lymphocytic choriomeningitis virus was found in 41 (3.5%) of 1,185 persons in the United States who had acute central nervous system disease or undifferentiated febrile illnesses. The results of analyses of antibody titers in paired serum samples suggest that a North American Tacaribe serocomplex virus was the causative agent of the illnesses in 2 persons and that lymphocytic choriomeningitis virus was the causative agent of the illnesses in 3 other antibody-positive persons in this study. The results of this study suggest that Tacaribe serocomplex viruses native to North America, as well as lymphocytic choriomeningitis virus, are causative agents of human disease in the United States.

The arenaviruses (family *Arenaviridae*, genus *Arenavirus*) known to occur in North America include Whitewater Arroyo virus (WWAV), 7 other members of the Tacaribe serocomplex (Table 1), and lymphocytic choriomeningitis virus (LCMV, the prototypic member of the lymphocytic choriomeningitis–Lassa serocomplex). Specific members of the order Rodentia are the principal hosts of the arenaviruses, for which natural host relationships have been well characterized. For example, the hispid cotton rat (*Sigmodon hispidus*) in Florida is the principal host of Tamiami virus (*6,7*), and the ubiquitous house mouse (*Mus musculus*) is the principal host of LCMV (*9*).

Five South American members of the Tacaribe serocomplex, LCMV, and Lassa virus are etiologic agents of severe febrile illnesses in humans (*10,11*). The human health significance of the North American Tacaribe serocomplex viruses has not been rigorously investigated (*12*).

Studies since the mid-1990s have shown that Tacaribe serocomplex viruses are widely distributed in the United States and Mexico and that woodrats (*Neotoma* spp.) and other members of the family Cricetidae are natural hosts of these viruses (*1–5,8,13,14*). The purpose of this study was to investigate whether humans have been infected with North American Tacaribe serocomplex viruses.

Materials and Methods

Samples of serum (n = 1,305), plasma (n = 2), and cerebrospinal fluid (n = 70) from 1,185 persons in the United States with acute central nervous system disease or undifferentiated febrile illnesses were tested for immunoglobulin (Ig) G against the WWAV prototype strain AV 9310135 and LCMV strain Armstrong by using an ELISA as described (*15*). The samples were diagnostic specimens submitted to the Arbovirus Diseases Branch, Division of Vector-Borne Infectious Diseases, Centers for Disease Control and Prevention (CDC) (Fort Collins, CO, USA) during 1989–2000 by public health laboratories in the United States. The samples had been tested selectively by CDC laboratorians for evidence of infection with St. Louis encephalitis virus, western equine encephalomyelitis virus, and other arthropod-borne agents of human disease. These tests had not yielded a specific diagnosis for any of the cases in this study.

Information about each case was limited to patient age, sex, date of illness onset, and state from which the samples were submitted. Most (634 [53.5%]) of the 1,185 case-patients were male. Ages at illness onset ranged from 0.2 months to 93 years (median 35 years), and 982 (82.0%) of the case-patients were ≥10 years of age at illness onset.

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Table 1. Natural hosts and geographic distribution of the North American Tacaribe serocomplex viruses

Virus	Natural host(s)	Location	Reference
Bear Canyon	Large-eared woodrat (*Neotoma macrotis*), California mouse (*Peromyscus californicus*)	California, USA	(*1*)
Big Brushy Tank	White-throated woodrat (*N. albigula*)	Arizona, USA	(*2*)
Catarina	Southern plains woodrat (*N. micropus*)	Texas, USA	(*3*)
Rio Catorce	White-toothed woodrat (*N. leucodon*)	San Luis Potosí, Mexico	(*4*)
Skinner Tank	Mexican woodrat (*N. mexicana*)	Arizona, USA	(*5*)
Tamiami	Hispid cotton rat (*Sigmodon hispidus*)	Florida, USA	(*6,7*)
Tonto Creek	White-throated woodrat (*N. albigula*)	Arizona, USA	(*2*)
Whitewater Arroyo	White-throated woodrat (*N. albigula*)	New Mexico, USA	(*8*)

The period between illness onset and sample collection ranged from 0 days to 10.1 years (median 31 days). At least 1 sample from each of 580 case-patients was collected before the end of week 4 of illness; for 108 case-patients multiple samples, representing different time points, were available. Cases were geographically distributed as follows: New England, 72 cases; Mid-Atlantic, 50; South Atlantic, 141; East North Central, 96; West North Central, 73; East South Central, 78; West South Central, 42; Mountain, 177; Pacific, 96; and unknown, 360.

A 1:80 dilution and 1:320 dilution of each sample was tested against the WWAV antigen, LCMV antigen, and corresponding comparison (negative-control) antigens. The adjusted optical density (AOD) of a sample-antigen reaction was the optical density of the well coated with the test antigen minus the optical density of the well coated with the corresponding control antigen. A sample was considered positive if the AOD at 1:80 was ≥0.250, the AOD at 1:320 was ≥0.250, and the sum of the AOD at 1:80 and AOD at 1:320 was ≥0.750. Endpoint titers against each antigen were measured in the positive samples by using serial 2-fold dilutions from 1:320 through 1:40,960. The antibody titer of a positive sample was the reciprocal of the highest dilution for which the AOD was ≥0.250. Titers <320 were 160 in comparisons of titers to WWAV and LCMV in individual samples. The apparent homologous virus in an antibody-positive sample was the virus associated with the highest titer if the absolute value of the difference between the titers to WWAV and LCMV was ≥4-fold.

Results

We detected antibody against an arenavirus in 41 (3.5%) of the 1,185 case-patients. Of the antibody-positive case-patients, most (27 [65.9%]) were male. Ages ranged from 4 years to 85 years (median 39 years). Antibody-positive samples were submitted from Florida, Massachusetts, and Wyoming (3 samples each) and Arizona, Idaho, Kansas, Maryland, Michigan New Mexico, New York, North Carolina, Ohio, Rhode Island, Tennessee, Washington, and Wisconsin (1 sample each). For 19 samples, state of submission was unknown.

Twelve persons had positive test results for WWAV but not LCMV; 28 for LCMV but not WWAV; and 1 for WWAV and LCMV (Table 2). In the positive samples, endpoint titers against WWAV and LCMV ranged from <320 to 10,240 and from <320 to 20,480, respectively. The apparent homologous virus was WWAV in 10, LCMV in 24, and indeterminate in 7 of antibody-positive persons (Table 2).

Ages of the 10 persons in whom WWAV was the apparent homologous virus ranged from 5 to 70 years (median 43 years). Samples from these persons were submitted from Arizona, New Mexico, and North Carolina (1 sample each) and Florida and Wyoming (2 samples each); for 3 samples, state of submission was unknown.

The ELISA included paired samples from 8 antibody-positive persons. Time from onset of illness to the first samples from these persons ranged from 0 to 47 days. In side-by-side tests, the endpoint titer to WWAV in the second sample was ≥4-fold higher than that to WWAV in the first sample in paired samples from 2 persons, and the endpoint titer to LCMV in the second sample was ≥4-fold higher than that to LCMV in the first sample in paired samples from 3 of the 6 other antibody-positive persons (Table 3).

Table 2. Antibody (immunoglobulin G) titers against WWAV and LCMV in 1,185 cases of acute central nervous system disease or undifferentiated febrile illnesses, United States*

No. cases	Antibody titer		Apparent homologous virus
	WWAV	LCMV	
5	640	<320	WWAV
1	1,280	<320	WWAV
3	2,560	<320	WWAV
1	10,240	<320	WWAV
7	<320	640	LCMV
3	<320	1,280	LCMV
5	<320	2,560	LCMV
4	<320	5,120	LCMV
2	<320	10,240	LCMV
3	<320	20,480	LCMV
2	320	<320	Indeterminate
1	640	1,280	Indeterminate
4	<320	320	Indeterminate
1,144	<320	<320	None

*WWAV, Whitewater Arroyo virus; LCMV, lymphocytic choriomeningitis virus.

Table 3. Antibody (immunoglobulin G) against WWAV and LCMV in paired serum samples from humans with acute central nervous system disease or undifferentiated febrile illnesses, United States*

Case-patient no.	Age, y, at illness onset	Days after illness onset		Antibody titer, WWAV		Antibody titer, LCMV		Apparent homologous virus
		S1	S2	S1	S2	S1	S2	
1	32	14	44	<320	640	<320	<320	WWAV
2	65	15	61	<320	2,560	<320	<320	WWAV
3	38	14	33	<320	<320	5,120	5,120	LCMV
4	51	2	68	<320	<320	320	20,480	LCMV
5	59	24	38	<320	<320	320	5,120	LCMV
6	72	0	15	<320	<320	<320	640	LCMV
7	12	25	33	<320	<320	320	320	Indeterminate
8	25	47	123	<320	<320	320	320	Indeterminate

*WWAV, Whitewater Arroyo virus; LCMV, lymphocytic choriomeningitis virus; S1, first sample; S2, second (last) sample in paired samples.

Discussion

Previously, antibody to Tamiami virus was found in 5 (3.8%) of 131 Seminole Indians sampled in southern Florida (*16*), and antibody to a Tacaribe serocomplex virus was found in 2 (0.24%) of 829 persons who had worked with cricetid rodents in North America (*15,17*). The results of our current study strengthen the notion that Tacaribe serocomplex viruses enzootic in North America are infectious in humans. The increase in antibody titer against WWAV in cases 1 and 2 in this study (Table 3) suggests that a North American Tacaribe serocomplex virus caused the illnesses in these persons.

The WWAV strain AV 9310135 was originally isolated from a white-throated woodrat (*N. albigula*) captured in northwestern New Mexico (*8*). A recent study demonstrated a high level of diversity among the amino acid sequences of the structural proteins of the North American Tacaribe serocomplex viruses (*5*). Hypothetically, human IgG against some North American Tacaribe serocomplex viruses is not strongly reactive against WWAV in ELISA. If so, the prevalence of antibody to Tacaribe serocomplex viruses in this study actually might be >3.5%.

The severity of human disease caused by LCMV ranges from mild febrile illness to severe encephalitis and disseminated disease (*18*). The results of this study suggest that the illnesses in case-patients 4–6 (Table 3) were caused by LCMV. Whether samples from these 3 persons were tested for anti-LCMV antibody (IgM or IgG) by clinical laboratories could not be determined from records maintained at CDC.

Specimens from 33 of the antibody-positive persons in this study were limited to single specimens. Perhaps these illnesses were caused by a North American Tacaribe serocomplex or by LCMV. The antibody titer to WWAV in the antibody-positive person from New Mexico was 10,240 in a serum sample collected on day 22 day after illness onset.

Future studies on the relevance to human health of the North American Tacaribe serocomplex viruses should include defining the clinical spectrum and epidemiology of human disease caused by these viruses. Some of these viruses may cause aseptic meningitis, encephalitis, or meningoencephalitis. Thus, human disease caused by North American Tacaribe serocomplex viruses may be confused with severe encephalitis caused by LCMV, especially in persons who report recent exposure to rodents.

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 4. 23	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		News release Alabama depertment of public health. Available from: http://www.adph.org/news/assets/110407.pdf	米国

研究報告の概要

○Serratia汚染原因の同定:速報
アラバマ州公衆保健局(ADPH)は2つの病院から、完全静脈栄養剤(TPN)を投与した患者に*Serratia marcescens*感染が生じたという通報を受けた。米国疾病管理予防センター(CDC)は、共通の原因として可能性のあるTPN製造業者を特定し、その業者からTPNを納入していた6つの病院を特定した。6つの病院で19症例(38歳～94歳;男性8名、女性11名)が報告された。
ADPHとCDCは、遺伝子解析結果、TPNを製造する際に使用していた容器とスターラー、容器をすすぐ為の水道栓及びTPNから分離された菌と、TPNを受けた入院患者12人から分離された*S.marcescens*が同じであったと確認した。さらにTPNの原料である混合アミノ酸1袋も、*S.marcescens*で汚染されていた。TPNを混合する時の殺菌工程の失敗が、汚染の原因になったと考えられる。この製造業者は通知を受け、汚染の可能性を情報提供し、生産を中止し、製品を回収した。
今のところ他の業者からTPN汚染の報告はなく、アラバマ州以外の病院にも当該製品は納入されていなかった。
ADPHは、CDCや他の機関とも協力し、*S.marcescens*感染発生の調査を続ける。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
アラバマ州公衆保健局と米国疾病管理予防センターは、6つの病院で発生した*Serratia marcescens*感染は、完全静脈栄養剤の製造工程での汚染が原因であると断定したとの報告である。	日本赤十字社では輸血による細菌感染予防対策として、すべての輸血用血液製剤を対象に、初流血除去及び保存前白血球除去を導入している。さらに、輸血情報リーフレット等により、細菌感染について医療機関へ情報提供し注意喚起しているほか、細菌感染が疑われる場合の対応を周知している。細菌の検出や不活化する方策について検討している。

(19)

NEWS RELEASE
ALABAMA DEPARTMENT OF PUBLIC HEALTH
RSA Tower 201 Monroe Street, Suite 914 Montgomery, AL 36104
Phone 334-206-5300 Fax 334-206-5534
www.adph.org

Likely source of Serratia contamination identified

FOR IMMEDIATE RELEASE

CONTACT:
Mary McIntyre, M.D.
(334) 206-5325

The Alabama Department of Public Health and the Centers for Disease Control and Prevention have determined that the *Serratia marcescens* bacteremia in 12 hospitalized individuals who received TPN (total parenteral nutrition) has the same genetic fingerprint as the organism isolated from a container and stirrer used to mix the powdered amino acids, from the tap water spigot used for rinsing the container, and from the TPN.

A bag of compounded amino acids used in the production of TPN has also grown *Serratia marcescens.* Genetic fingerprint results are pending on the compounded amino acids.

The Alabama Department of Public Health is aware of 19 cases of *Serratia marcescens* in patients in six Alabama hospitals. Of these cases, 12 samples from individuals were matched with the bacterium found at Meds IV Pharmacy in Birmingham. Of the remaining seven cases in question, six have no samples available to test for a genetic match and one case is pending.

A failure in a step of the sterilization process in the compounding of TPN was most likely the cause of contamination. Use of these contaminated products led to a bacterial bloodstream infection in these 19 patients.

On March 16, ADPH was notified that an outbreak had occurred in two hospitals among patients receiving TPN. CDC's initial investigation identified TPN produced by Meds IV as a potential common source and determined that six hospitals received TPN from this pharmacy.

Illness with *Serratia marcescens* bacteremia occurred in approximately 35 percent of patients receiving TPN from Meds IV during March. Seventeen cases were reported in March, and two additional cases were retrospectively identified during the investigation, one in January and one in February.

The individuals affected are in the age range from 38 to 94 years; 8 males and 11 females were infected. The numbers of cases and deaths by hospital are as follows: Baptist Princeton, 7 cases, 4 deaths; Baptist Shelby, 5 cases, 2 deaths; Medical West, 3 cases, 1 death; Cooper Green Mercy, 1 case, no deaths; Baptist Medical Center Prattville, 1 case, 1 death; and Select Specialty Hospital of Birmingham, 2 cases, 1 death.

Meds IV was notified and informed its customers of the possibility of contamination. ADPH has been informed that impacted hospitals immediately stopped using TPN received from this

pharmacy and that the pharmacy discontinued all production. On March 24, Meds IV recalled all of its IV compounded products.

ADPH continues an ongoing investigation of the outbreak of *Serratia marcescens* bacteremia in collaboration with the CDC, the U.S. Food and Drug Administration, the Alabama Hospital Association, and the State Board of Pharmacy. At this time, there have been no reports of contaminated TPN, from any other pharmacy, sent to hospitals in Alabama or any other state.

As more information becomes available, ADPH will provide updates.

-30-

4/7/11

別紙様式第2-1

No. 27

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 6. 20	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	Eurosurveillance Vol.16 Is.24	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			ドイツ

研究報告の概要

○志賀毒素／ベロ毒素産生大腸菌感染による出血性下痢及び溶血性尿毒症症候群の大規模アウトブレイク発生時における強化サーベイランス、ドイツ、2011年5月～6月

ドイツでは感染症のための広範な法定サーベイランスシステムが確立している。しかしこのシステムは、患者情報が地域レベルから州や国レベルに報告されるまでに時間を要する。

2011年5月19日、ドイツのロベルト・コッホ研究所は、志賀毒素／ベロ毒素産生大腸菌(STEC／VTEC) O104:H4感染による溶血性尿毒症症候群(HUS)患者がハンブルクで多数発生しているとの報告を受け、翌日当該地域に調査チームを派遣した。患者の急激な増加を受け、強化サーベイランスが必要であることが確認された。このため、以下のような変更を行った。

疫学情報交換の集約化／国レベルまでの情報伝達の迅速化／病院の救急部における出血性下痢症の症候群サーベイランスシステムの導入／ドイツにおけるHUS治療受け入れ能力の評価／検査機関でのアクティブサーベイランスの開始

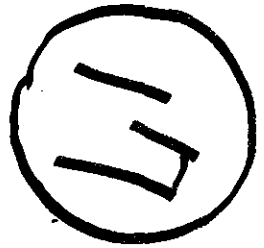
これらの追加サーベイランスシステムは自主的に行われたものであり、今回のアウトブレイクにおいて、より迅速なモニタリングを可能にした。サーベイランスの強化により、確定患者の実数把握や国際援助が必要かどうかの判断、新規患者発生の発生動向について把握することができた。しかし、法定サーベイランスシステムにおける情報伝達速度については迅速化する必要がある。また新規患者の発生傾向を迅速に探知するため、この先少なくとも3カ月間は病院の救急部における症候群サーベイランスの継続を推奨する。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
志賀毒素／ベロ毒素産生大腸菌感染症による出血性下痢及び溶血性尿毒症症候群の大規模アウトブレイクが発生した際に強化サーベイランスを実施し、通常のサーベイランスに比べてより迅速にモニタリングを行うことができたとの報告である。	今後も国内外の感染症アウトブレイクに関する情報の収集に努める。



RAPID COMMUNICATIONS

Enhanced surveillance during a large outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing *Escherichia coli* in Germany, May to June 2011

M Wadl (WadlM@rki.de)[1], T Rieck[1,2], M Nachtnebel[1,3,4], B Greutélaers[1,3,4], M an der Heiden[1], D Altmann[1], W Hellenbrand[1], M Faber[1], C Frank[1], B Schweickert[1], G Krause[1], J Benzler[1], T Eckmanns[1], on behalf of the HUS surveillance and laboratory team[5]

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5. The members of the team are listed at the end of the article

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Euro Surveill. 2011;16(24):pii=19893. Available online: http://www.eurosurveillance.org/ViewArticle.aspx?ArticleId=19893

Article published on 16 June 2011

Germany has a well established broad statutory surveillance system for infectious diseases. In the context of the current outbreak of bloody diarrhoea and haemolytic uraemic syndrome caused by Shiga toxin/verotoxin-producing *Escherichia coli* in Germany it became clear that the provisions of the routine surveillance system were not sufficient for an adequate response. This article describes the timeline and concepts of the enhanced surveillance implemented during this public health emergency.

On Thursday, 19 May 2011, the Robert Koch Institute (RKI) was informed about a cluster of cases of haemolytic uremic syndrome (HUS) due to Shiga toxin/verotoxin-producing *Escherichia coli* (STEC/VTEC) O104:H4 in the area of Hamburg, Germany. An RKI investigation team visited the affected area the following day. In the face of rapidly rising case numbers, a need for enhanced surveillance was identified on 23 May. We describe here the timeline and concepts of the enhanced surveillance implemented during this massive outbreak of bloody diarrhoea and HUS in May and June 2011 in Germany.

Routine surveillance system

In Germany, STEC/VTEC and HUS have been statutorily notifiable since 2001 according to the Protection against Infection Act (Infektionsschutzgesetz, IfSG [1]). While STEC/VTEC surveillance is based on laboratory analyses, HUS surveillance relies on physicians. Heads of laboratories and physicians must report cases to the local health authorities within 24 hours. The incoming data is validated by the local health authorities and documented electronically. Cases fulfilling the surveillance case definition as issued by RKI [2] are transmitted in anonymous form to the state health authorities by the third working day of the following week. The state health authorities again validate incoming cases and transmit the data to the RKI within the following week. Hence, transferring information on a case from the local to the national health authority may take from a few days up to 16 days.

Epidemiological information is fed back from RKI at least weekly to the stakeholders, e.g. responsible authorities, physicians and laboratories. Information exchange includes teleconferences, reports in the RKI's weekly *Epidemiological Bulletin* and the internet database SurvStat [3].

Enhanced surveillance system

In the context of the outbreak it became immediately clear that the provisions of the routine surveillance system were not sufficient for an adequate response. Hence, the following amendments were implemented:

- Centralising the epidemiological information exchange,
- Accelerating the data flow to the national level,
- Implementing a syndromic surveillance system for bloody diarrhoea in emergency departments,
- Assessing the capacities for HUS-treatment in Germany,
- Initiating active laboratory surveillance.

An overview of routine and newly implemented surveillance systems is given in Figure 1.

Centralising the epidemiological information exchange

On 23 May 2011, the 'Lagezentrum' at the RKI was activated as a central emergency operations centre. A large number of RKI staff was involved in coordinating the collection of epidemiologic information and organising the public health response. From 23 May onwards, teleconferences were conducted almost daily with the responsible state, national and international authorities. Starting on 24 May, epidemiological reports were distributed daily to the responsible authorities, physicians and laboratories to feed back relevant information. Several outbreak-related articles were published in *Eurosurveillance* [4,5] and the German *Epidemiological Bulletin*. The public was regularly informed about the outbreak situation via the RKI website starting on 23 May, press releases were issued on 3 and 10 June. The Federal Centre for Health Education (Bundeszentrale für Gesundheitliche Aufklärung, BZGA), has provided outbreak-related public health advice to the public since 24 May.

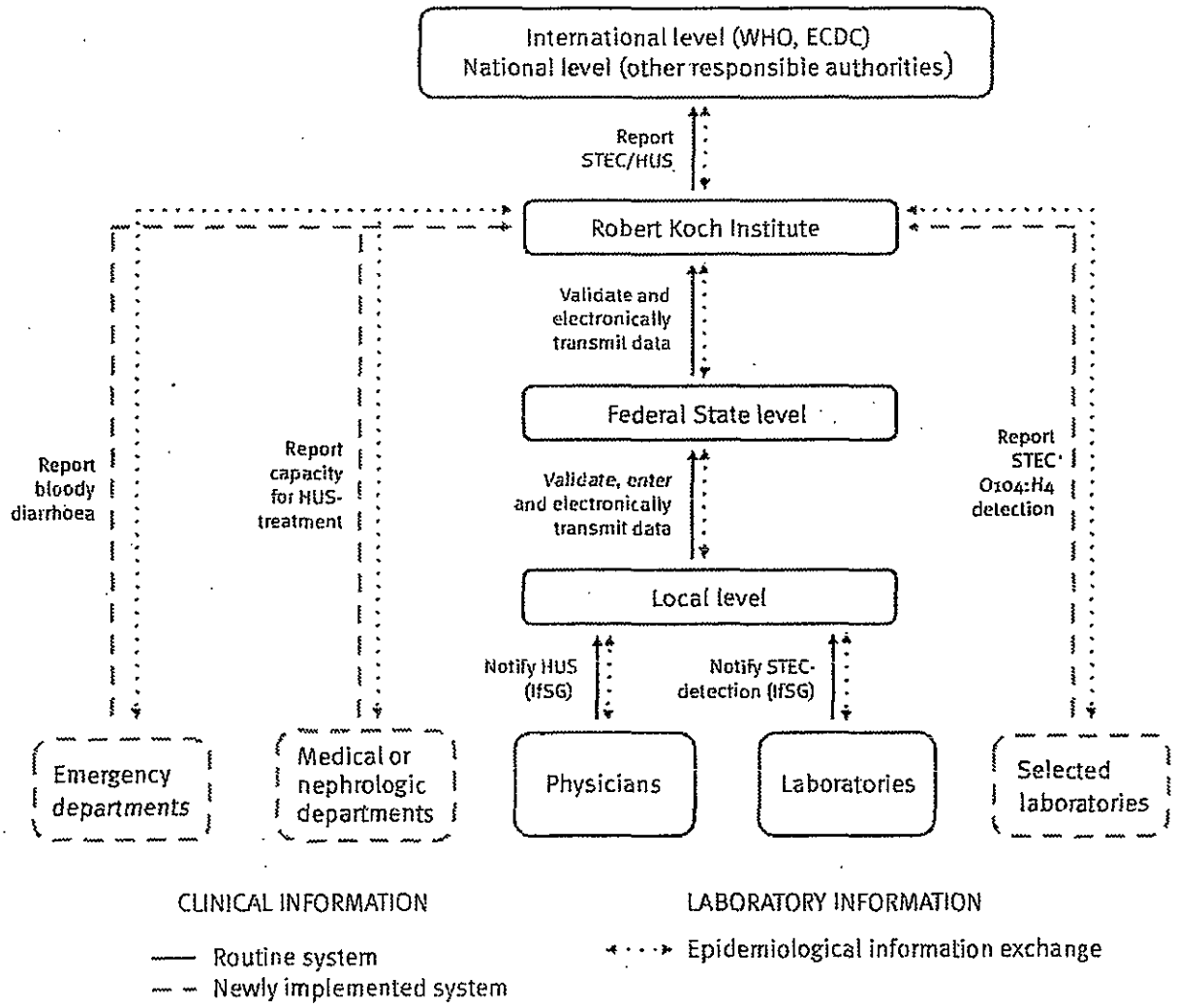
Accelerating the data flow to the national level

From 23 to 27 May 2011, state health authorities were asked to transmit aggregated data via email on a daily basis to the RKI. Concurrently, health authorities were urged to enter and transmit the IfSG data via the electronic surveillance system daily, so that case by case reporting could overtake the aggregated reporting on 27 May. A specific reporting form was published on 26 May to facilitate notification of HUS cases by physicians.

In addition, the existing RKI surveillance case definition was adapted to the outbreak situation to ensure systematic data collection. Modifications included

FIGURE 1

Data and information flow to and from the Robert Koch Institute during the period of enhanced surveillance, STEC/HUS outbreak, Germany, spring 2011



ECDC: European Centre for Disease Prevention and Control; HUS: haemolytic uraemic syndrome; IfSG: German Protection against Infection Act; STEC: Shiga toxin-producing *Escherichia coli*; WHO: World Health Organization.

limitations of time (onset of disease from 1 May 2011), place (epidemiological link to Germany) and person (e.g. consumption of a food item that was acquired in Germany) concerning exposure as well as inclusion of suspected cases [6].

One challenge was counting outbreak-related cases of STEC/VTEC O104:H4 separately from other STEC/VTEC cases, of which a mean of 992 cases annually had been reported to the RKI between 2001 and 2010. In the absence of comprehensive laboratory data for a majority of reported cases, the case definition was revised in a way that listed as exclusion criteria all specific laboratory test results that were not consistent with the characteristics of the outbreak strain.

As of June 12, a total of 3,228 STEC/VTEC and HUS cases in Germany have been associated with the outbreak (Figure 2). The majority of cases (51%) fell ill between 18 and 25 May. The place of exposure was suspected to lie in north-western parts of Germany for most cases (Figure 3). Of the 781 reported HUS cases, 69% were female and 88% were 20 years of age or older. Overall, 22 notified HUS cases have died. Among all 2,447 STEC/VTEC cases, 59% were female and 87% were 20 years of age or older. Thirteen notified STEC/VTEC cases have died.

Figure 4 shows the transmission delay in days from the local to the national level during the STEC/HUS outbreak period among HUS cases. Among the 740 HUS cases (96%) with known date of notification to the local health authorities, the median transmission delay was two days (25th–75th percentile: 1–4 days, minimum–maximum: 0–18 days). The first HUS-case was reported to the RKI through the electronic surveillance system on 18 May. Another three HUS cases were reported on 23 May. Thereafter, the accelerated data flow became evident, for instance, 47 HUS cases were reported to the RKI on 24 May, 50 HUS cases on 25 May, 100 HUS cases on 26 May and 116 HUS cases on 27 May.

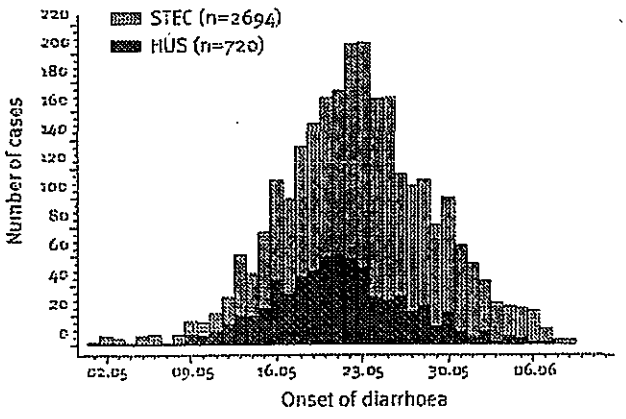
Implementing a syndromic surveillance system for bloody diarrhoea in emergency departments

Since STEC patients often present with bloody diarrhoea, emergency departments (ED) constitute appropriate facilities for the assessment of the temporal trend of an STEC-outbreak. We implemented the surveillance of patients with and without bloody diarrhoea in ED on 27 May.

Participating ED were located in all federal states of Germany, both in areas affected and not affected by the STEC/HUS outbreak (see Figure 4). Data collection covered the total number of new patients in participating ED and the number of patients presenting with bloody diarrhoea by sex and age group (<20 years, ≥20 years). The data were transferred to the RKI by email or fax every day.

FIGURE 2

Reported STEC/VTEC and HUS cases, by date of onset of diarrhoea*, Germany, May–June 2011 (n=2,694)

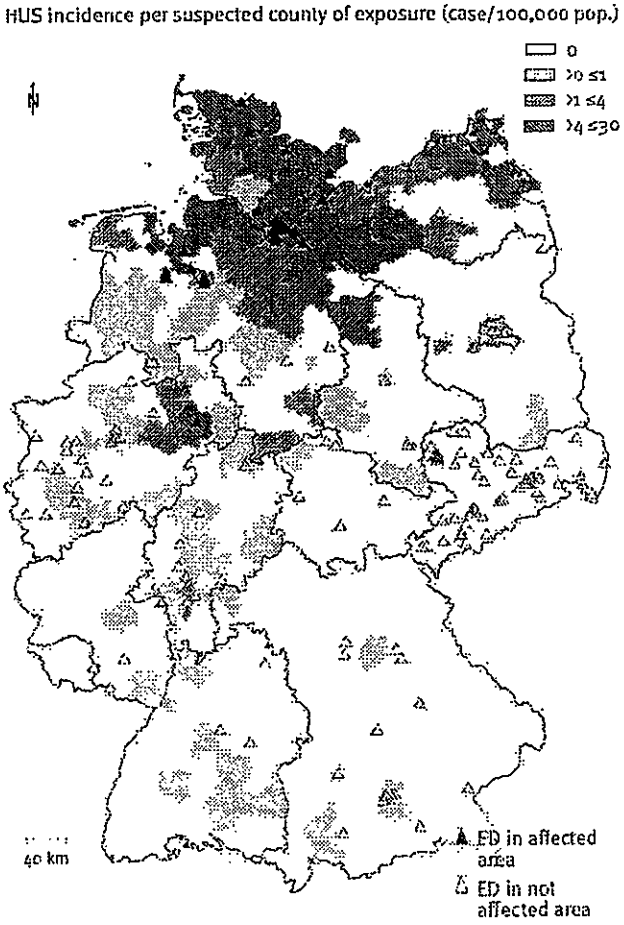


ED: Emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

* Only cases with a notified date of onset since 1 May 2011.

FIGURE 3

Cumulative incidence of HUS cases per suspected county of exposure and emergency departments actively participating in the syndromic surveillance system, Germany, May–June 2011

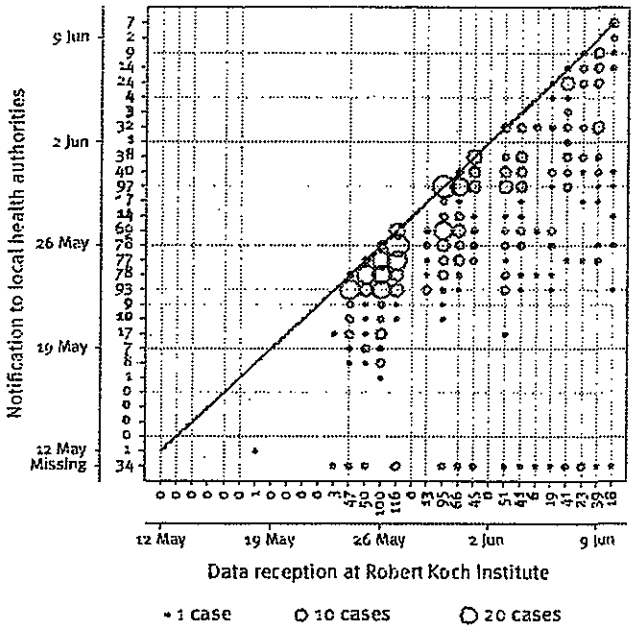


ED: Emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

As of 12 June, a total of 174 ED have participated in the syndromic surveillance system; 27 of which were located within affected areas. The number of ED actively reporting varied from day to day. Thus results may change as further, re¬tro¬spective, reports are received from ED. Between 28 May and 12 June, 4.7% (744/15,884) of all patients presenting to ED in affected regions were reported as having bloody diarrhoea (Figure 5); this proportion was 0.8% (464/55,255) in non-affected regions. Figure 5 shows the sex and age distribution of patients with BD as well as the number of participating ED in affected areas. Women were affected more often than men, with a decreasing proportion of female cases observed after 30 May. Since 6 June, the proportion of all patients with bloody diarrhoea among the patients presenting to emergency departments has remained on an average of 3.6%.

FIGURE 4

Date of notification of HUS cases to local health authority in relation to date of reception at Robert Koch Institute, Germany, May–June 2011



HUS: haemolytic uraemic syndrome; PH: public holiday; STEC: Shiga toxin-producing *Escherichia coli*; WE: weekend.

Weekends and public holidays in bold; the x- and y-axis additionally show the number of reports received.

The size of the circle is equivalent to the number of cases (examples for 1, 10 and 20 cases shown in the legend).

Assessing the capacities for treatment of haemolytic uraemic syndrome in Germany

From 30 May onwards, the German Society for Nephrology collected data on the HUS treatment capacities in Germany and reported these regularly via e mail to the RKI. During the outbreak period, 79 hospitals, located in 15 of the 16 federal states, provided almost daily information: all but two confirmed having sufficient capacities for treating HUS patients.

Initiating active laboratory surveillance

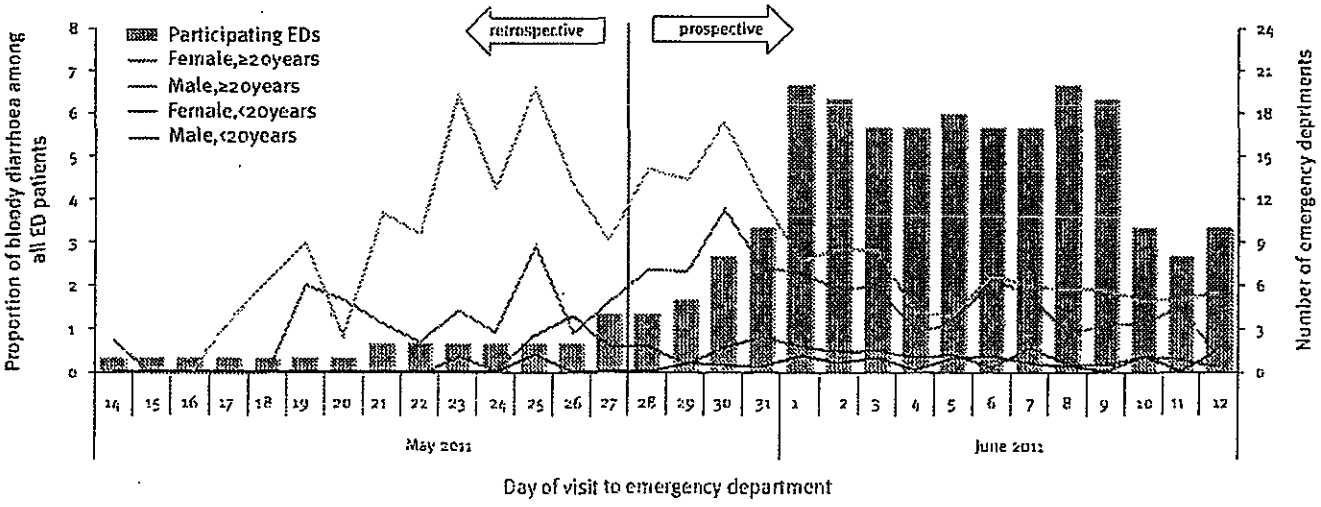
Since 25 May, the RKI has asked four laboratories for daily data transfer per email or telephone. As of 12 June, a total of 195 (6%) of all 3,228 STEC/HUS cases have been confirmed through the routine mandatory system as caused by the outbreak strain STEC/VTEC O104, whereas the active system provided evidence that at least 335 patient samples were related to the outbreak strain.

Reports to the European Union and the World Health Organization

Following international law, Germany informed the European Union (EU) of the STEC/HUS outbreak via

FIGURE 5

Proportions of patients with bloody diarrhoea among all patients presenting to emergency departments, by age and sex, in areas affected by the STEC/HUS outbreak, Germany, May–June 2011 (n=744)



D: emergency department; HUS: haemolytic uraemic syndrome; STEC: Shiga toxin-producing *Escherichia coli*.

the Early Warning and Response System (EWRS) on 22 May 2011, and notified the event as a potential public health emergency of international concern within the framework of the International Health Regulations (IHR) 2005 on 24 May. The RKI sent updates on the situation to EWRS, the Epidemic Intelligence Information System (EPIS) and the World Health Organization (WHO) on a daily basis.

Both the European Centre for Disease Prevention and Control (ECDC) and the WHO immediately supported the outbreak investigations by staying in close contact with Germany and other countries and reporting imported STEC/HUS cases (in travellers) associated with the outbreak.

Conclusions

Germany has a well established broad statutory surveillance system for infectious diseases. However, the rather long time limits permitted for communicating information on cases from the local to the state/national level led to delayed recognition of this outbreak: The first report at the national level was received on 18 May 2011, while the first outbreak-associated cases fell ill on 1 May, with a sharp increase in case numbers on 9 May. This is a limitation requiring further evaluation. In this specific outbreak situation, the mandatory surveillance system required enhancement that was rapidly and effectively implemented. Physicians, laboratories, local and state health authorities supported the acceleration and extension of the system extraordinarily well. Feedback to the public, the responsible authorities, physicians and laboratories was ensured daily, e.g. by updates on websites, teleconferences and reports.

The additional surveillance instruments were voluntary and allowed for more timely monitoring of this public health emergency. Laboratory surveillance permitted assessment of the actual number of laboratory-confirmed outbreak cases particularly in the early stages. Monitoring capacity for treating HUS patients in German hospitals allowed us to evaluate whether or not international help would be needed. Syndromic surveillance in ED permitted us to follow the temporal trend of bloody diarrhoea patients as a proxy for potentially new STEC/VTEC cases.

We conclude that infectious disease surveillance in Germany can rapidly be adapted to specific outbreak situations. Nevertheless, data flow within the statutory surveillance system should be accelerated, e.g. by use of an electronic notification system by physicians and laboratories, and a common central data base. We recommend continuing syndromic surveillance in ED for at least the next three months to ensure timely detection of possible new trends.

Acknowledgements

We gratefully acknowledge the contribution of all physicians and laboratories as well as the local and state health departments, whose investigations and notifications were the data basis of this report. We especially thank the various emergency departments participating in the syndromic surveillance system, the laboratories involved in the laboratory surveillance system and the German Society for Nephrology for their support.

HUS surveillance and laboratory team

Muna Abu Sin, Susanne Behnke, Bonita Brodhun, Hermann Claus, Yvonne Delere, Lena Fiebig, Barbara Gunsenheimer-Bartmeyer, Andreas Gilsdorf, Karin Haar, Barbara Hauer, Margareta Ung-Zu Kang, Monika Luchtenberg, Inge Mücke, Janina Neifer, Ines Noll, Susanne Schink, Irene Schöneberg, Ed Velasco, Bettina Weiß.

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医薬品
医薬部外品　　研究報告　調査報告書
化粧品

識別番号・報告回数			報告日	第一報入手日 2011年8月19日	新医薬品等の区分 該当なし	厚生労働省処理欄

一般的名称	①②乾燥抗HBs人免疫グロブリン ③ポリエチレングリコール処理抗HBs人免疫グロブリン	研究報告の公表状況	CDC／MMWR 60 (32) 1083-1086/2011/08/19	公表国 アメリカ
販売名 （企業名）	①ヘブスブリン筋注用200単位　（ベネシス） ②ヘブスブリン筋注用1000単位　（ベネシス） ③ヘブスブリンIH静注1000単位　（ベネシス）			

研究報告の概要

重篤な、時に致命的な肺炎になるレジオネラ病(LD)と、インフルエンザ様の定型的疾患のポンティアック熱(PF)は、レジオネラ菌によって引き起こされるレジオネラ症の二つの最も一般的な症状である。レジオネラ症例は、国立届出疾病監視システム(NNDSS)、及び旅行関連症例の監視データを管理、集団発生の検出を強化するために設計したレジオネラ疾病監視システム(SLDSS)を通じてCDCに報告された。この報告書は、米国50州とコロンビア特別区(DC)から2000年～2009年の間NNDSSへ報告された症例を評価し、10万人当たりの年齢補正した発生率を計算した。米国のレジオネラ症の年間報告数は2000年の1,110から2009年の3,522（2000年から2009年の間、NNDSSへ22,418例のレジオネラ症が報告された）へ217%増加し、粗い国内発生率は2000年の0.39/10万人から2009年の1.15/10万人へ192%増加した。

報告企業の意見	今後の対応
レジオネラは、レジオネラ属に属する真正細菌の総称であり、2～5μm位の好気性グラム陰性の桿菌で、一本以上の鞭毛を持っている。万一、原料血漿にレジオネラ菌が混入したとしても、除菌ろ過等の製造工程にて除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリンIH静注1000単位の記載を示す。

2. 重要な基本的注意

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

8

Centers for Disease Control and Prevention
CDC 24/7: Saving Lives. Protecting People. Saving Money through Prevention.

Morbidity and Mortality Weekly Report (*MMWR*)

Legionellosis --- United States, 2000--2009

Weekly
August 19, 2011 / 60(32);1083-1086

Legionnaires disease (LD), a serious, sometimes lethal pneumonia, and Pontiac fever (PF), an influenza-like, self-limited illness, are the two most common forms of legionellosis, which is caused by *Legionella* bacteria. Legionellosis cases are reported to CDC through the National Notifiable Disease Surveillance System (NNDSS) and a Supplemental Legionnaires Disease Surveillance System (SLDSS) designed to manage surveillance data on travel-related cases and enhance outbreak detection. For this report, cases reported to NNDSS during 2000--2009 from the 50 states and the District of Columbia (DC) were assessed, and crude and age-adjusted incidence rates per 100,000 persons were calculated. U.S. legionellosis cases reported annually increased 217%, from 1,110 in 2000 to 3,522 in 2009, and the crude national incidence rate increased 192%, from 0.39 per 100,000 persons in 2000 to 1.15 in 2009. Because NNDSS is a passive surveillance system dependent on health-care providers and laboratories reporting cases, the actual incidence of legionellosis in the United States likely is higher. Although NNDSS does not record legionellosis cases by type, 99.5% of the legionellosis cases reported to SLDSS during 2005--2009 were classified as LD and 0.5% as PF. Legionellosis surveillance was added to the population-based Active Bacterial Core surveillance (ABCs) system in January 2011 to assess reasons for these increases in numbers of reported cases. The rise in reported cases reinforces the need for health-care providers in all parts of the United States to test and treat adults with severe community-acquired pneumonia for LD, to be vigilant for health-care--associated LD, and to report legionellosis cases to public health authorities.

NNDSS receives reports of cases of nationally notifiable diseases from state health departments, including data on case demographics, the earliest date associated with the patient's illness in public health records (i.e., the date of symptom onset, date of diagnosis, date of confirmatory laboratory test, or the date of the report of the case to the county or state, whichever is earliest), the date of report to CDC, the case status (i.e., confirmed, probable, or suspected), and whether or not the case is part of an outbreak. NNDSS data for 2000--2009 were used to describe legionellosis case demographics, assess seasonal patterns of legionellosis infection, and, using denominators from the 2000 U.S. standard population (*1*) and U.S. Census Bureau estimates, calculate crude and age-standardized incidence rates for the entire United States (excluding U.S. territories) and for each of the nine U.S. Census divisions.* Only cases considered confirmed under the 2005 Council of State and Territorial Epidemiologists' (CSTE) legionellosis case definitions are described in this report.† To be classified as confirmed, cases must be clinically compatible with legionellosis (i.e., fever, myalgia, cough, and/or clinical or radiographic evidence of pneumonia) and meet at least one of the confirmatory laboratory criteria (i.e., recovery of *Legionella* sp. in culture, detection of *Legionella pneumophila* serogroup 1 antigen in urine, or fourfold or greater rise in *L. pneumophila* serogroup 1-specific serum antibodies).

States also are encouraged to report cases to SLDSS to enhance detection of travel-related outbreaks and to provide information on additional legionellosis case variables not captured by NNDSS.§ Legionellosis cases ideally should be reported to both NNDSS and SLDSS. SLDSS collects information related to case demographics, diagnosis, diagnostic testing, hospitalization, outcome,

outbreak involvement, nosocomial classification, and recent travel history. In addition to the reports of cases among U.S. residents received from state health departments, SLDSS reports occasionally come from cruise lines, health-care providers, and private citizens. Those additional reports are verified with the relevant state health departments before inclusion in the SLDSS database. Foreign public health authorities also report cases to SLDSS, usually among travelers to the United States. In 2005, CSTE issued a position statement (2) requesting that all legionellosis cases be reported to SLDSS, but such reporting is not mandatory, and case follow-up varies by state and county based on staffing availability and perceived public health importance. For this report, SLDSS data were used to characterize diagnoses, diagnostic testing, outcomes, outbreak involvement, and recent travel.¶ Because of potential differences in data received by SLDSS before and after the 2005 CSTE position statement, separate analyses were conducted using cases with onset during 2000--2009 (NNDSS data) and 2005--2009 (SLDSS data).

During 2000--2009, the 50 states and DC reported 22,418 cases of legionellosis to NNDSS. The crude national incidence rate increased 192%, from 0.39 per 100,000 persons in 2000 to 1.15 in 2009, and the age-adjusted incidence of legionellosis in the United States increased 170%, from 0.40 to 1.08 cases per 100,000 persons. In 2000, the age-adjusted incidence varied substantially by U.S. Census division, from 0.09 cases per 100,000 persons in the West South Central division to 0.73 cases in the Middle Atlantic division. This disparity increased absolutely over the decade (Middle Atlantic division: 2.60 cases per 100,000 persons and West South Central division: 0.44 cases in 2009) (Table 1). All reporting divisions had an increase in age-adjusted legionellosis incidence from 2000--2001 to 2008--2009, ranging from a 101% increase in the West North Central division to 294% in the West South Central division. Nationally, 16,595 cases (74%) were in persons aged ≥50 years, and 14,255 (64%) persons were male (Table 2). Legionellosis incidence increased for all age groups from 2000 to 2009, ranging from 8% for persons aged ≤9 years to 287% for persons aged ≥80 years.

Among the 18,392 cases (82%) reported to NNDSS with available information on race, 78% were white, 19% were black, and 3% were American Indian/Alaska Native, Asian, or other (Table 2).** Cases tended to occur in the summer and early fall, with the June--October period accounting for 62% of the cases reported each year (Figure).

During 2005--2009, a total of 5,080 confirmed legionellosis cases among U.S. residents were reported to SLDSS by 47 states,†† accounting for 35% of the 14,554 confirmed cases reported to NNDSS during the same period by all 50 states and DC. An additional 82 confirmed legionellosis cases were reported among foreign visitors to the United States. A total of 1,220 (24%) cases involving U.S. residents were travel-associated; 81% of these involved domestic travel only, and 5% involved cruise ship travel. Travel-associated cases accounted for at least two thirds of the cases reported to SLDSS from 21 states, 11 of which reported only travel-associated cases, suggesting a bias against reporting nontravel-associated cases to SLDSS from some states. Of 3,872 (76%) U.S. resident cases with data available, 4% were associated with a known legionellosis outbreak or possible cluster. Information on clinical outcomes was available for 4,478 (88%) U.S. resident cases, 8% of which resulted in deaths. Urine antigen tests were used to confirm 97% of U.S. resident cases reported during 2005--2009. Only 5% of cases were confirmed by culture during this period, and <1% were confirmed by either serologic or direct fluorescent antigen testing.

Reported by

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Editorial Note

Reported legionellosis incidence rates increased nearly threefold during 2000--2009. In 2009, NNDSS received 3,522 case reports, the most since legionellosis became a reportable disease in 1976 (*3,4*). Increased rates were observed across all age groups and geographic regions. The reported case totals likely underestimate the actual disease burden; the most recent completed U.S. population--based pneumonia etiology study estimated that 8,000--18,000 persons are hospitalized each year with LD (*5*).

An increasing population of older persons contributed to the increase in reported legionellosis cases. Other factors that might have contributed include an increasing population of persons at high risk for infection; improved diagnosis and reporting, possibly stimulated by the 2005 CSTE endorsement of more timely and sensitive legionellosis surveillance; and increased use of urine *Legionella* antigen testing. However, because increases in urine antigen testing began in the 1980s, its use is unlikely to account for the entire increase in legionellosis cases since 2000 (*3,4*).

Urine antigen tests are easy to perform and provide timely, accurate results (sensitivity: 60%--80%; specificity: >99%) for detecting *L. pneumophila* serogroup 1, the causative agent in 70%--80% of LD cases (*6*). In contrast, culture of respiratory samples from possible LD cases (sensitivity: 20%--80%; specificity: >99%) can detect all forms of *Legionella* but has a lengthy turnaround time, and its sensitivity is highly dependent on the skill of laboratory personnel. Similarly, identifying legionellosis through paired serology (sensitivity: 70%--80%; specificity: >95%) involves substantial logistical challenges, whereas direct fluorescent antigen testing for LD (sensitivity: 25%--75%; specificity: >95%) can be technically demanding and can result in false positives resulting from cross-reactions with other bacteria. Only urine antigen and serology are useful for detecting PF, but the sensitivity of these tests for confirmation of PF is substantially lower than for LD (*7*).

Similar to the findings of previous studies, males accounted for >60% of cases, and increasing age was a major risk factor for legionellosis (*3,4*). However, the finding that blacks accounted for a disproportionately high number of cases relative to their 12% share of the population was unexpected. Insufficient information is available to confirm whether these patterns might be the result of differences in underlying risk factors or exposures to *Legionella*, and the high proportion of cases in persons of unknown race limits the interpretation of the racial differences observed.

Legionellosis demonstrates seasonal and geographic variability. During 2000--2009, nearly all regions reported their highest proportion of cases during the summer and early fall. The reported 2009 age-adjusted legionellosis rate in the Middle Atlantic division was nearly six times higher than the rate in the West South Central division. Whether these differences are related to the frequency of testing or reporting is unclear; nonetheless, clinicians should be particularly vigilant for possible LD during the summer and early fall and in geographic areas of relatively high legionellosis incidence. Although use of a urine antigen test for *Legionella* is recommended for cases of severe community-acquired pneumonia (*8*), collection of respiratory specimens for *Legionella*-specific culture also is encouraged as a means to detect all species and subgroups of *Legionella* and enable strain identification in the event of an outbreak. Urine antigen tests and *Legionella*-specific culture also are recommended for suspected cases of health-care--associated LD (*9*).

The findings in this report are subject to at least four limitations. First, current passive surveillance systems cannot determine whether the observed increase in legionellosis cases is actual or an artifact of improved detection or reporting. Second, surveillance likely is biased toward capture of more severe LD cases that are more likely to be tested for *Legionella*, missing those that have been empirically treated with antibiotics active against *Legionella* spp. and those not requiring hospitalization. Third, the nonspecific symptoms of and lack of good diagnostic tests for PF likely result in substantial underdiagnosis of this form of legionellosis. Finally, the proportion of cases that are potentially travel-associated likely is an overestimate resulting from a bias in many states toward primarily reporting travel-associated cases to SLDSS.

A better understanding of the disease burden and the epidemiology of legionellosis is important, but current passive surveillance systems cannot provide all the information required. In January 2011, active laboratory-based and population-based surveillance was launched in 10 ABCs sites around the country.§§ Data from this surveillance will be used to obtain population-based estimates of disease incidence; further describe demographic, seasonal, and geographic variability; and evaluate and improve legionellosis prevention efforts, such as the guidance provided by the American Society of Heating, Refrigerating, and Air Conditioning Engineers on preventing legionellosis associated with building water systems (*10*).

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* *New England:* Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont; *Middle Atlantic:* New Jersey, New York, and Pennsylvania; *East North Central:* Illinois, Indiana, Michigan, Wisconsin, and Ohio; *West North Central:* Iowa, Kansas, Missouri, Minnesota, Nebraska, North Dakota, and South Dakota; *South Atlantic:* Delaware, District of Columbia, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, and West Virginia; *East South Central:* Alabama, Kentucky, Mississippi, and Tennessee; *West South Central:* Arkansas, Louisiana, Oklahoma, and Texas; *Mountain:* Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming; *Pacific:* Alaska, California, Hawaii, Oregon, and Washington.

† The previous case definition, in use during 1996--2004, also included detection of *Legionella pneumophila* serogroup 1 through direct fluorescent antibody testing as a confirmatory laboratory test. The 2005 case definition is available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/legionellosis_current.htm. The 1996 case definition is available at http://www.cdc.gov/osels/ph_surveillance/nndss/casedef/legionellosis1996.htm.

§ A legionellosis case report form is available to state and local health departments at http://www.cdc.gov/legionella/files/legionella_case_report.pdf.

¶ A case of legionellosis was considered to be potentially travel-associated if the patient reported spending at least one night away from home during the 2 weeks before illness onset.

** Data on the ethnicity of cases reported to NNDSS were not included because 39% of cases were in persons of unknown ethnicity.

†† Nebraska, North Dakota, Wyoming, and DC did not report any confirmed legionellosis cases to SLDSS during 2005--2009. During this period, 29--40 states reported cases to SLDSS each year.

§§ Additional information is available at http://www.cdc.gov/abcs/index.html.

What is already known on this topic?

Thousands of cases of legionellosis occur each year in the United States as either Legionnaires disease, an often severe form of pneumonia, or Pontiac fever, an influenza-like, self-limited illness.

What is added by this report?

The incidence of reported legionellosis in the United States nearly tripled during 2000--2009, from 0.39 per 100,000 persons to 1.15. The reasons for this increase are unknown, but increases in the number of older persons and persons at high risk for infection and increased case detection or reporting might have played a role. Incidence increased with age and was highest in the Northeast.

What are the implications for public health practice?

Active, population-based legionellosis surveillance is needed to better assess the epidemiology and apparently increasing incidence of legionellosis in the United States. The rise in reported cases reinforces the need for health-care providers to test adults with severe community-acquired pneumonia or health-care--associated pneumonia for Legionnaires disease and report legionellosis cases to public health authorities.

TABLE 1. Age-adjusted incidence of reported legionellosis cases, by U.S. Census division* and year, 2000--2009

U.S. Census division	Annual incidence per 100,000 population									
	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
New England	0.38	0.48	0.81	0.79	0.71	1.00	1.20	1.04	1.43	1.21
Middle Atlantic	0.73	0.67	0.88	1.41	1.25	1.74	2.21	1.86	2.33	2.60
East North Central	0.64	0.68	0.64	0.97	1.03	0.96	1.26	1.24	1.34	1.44
West North Central	0.35	0.27	0.33	0.37	0.38	0.49	0.40	0.54	0.66	0.60
South Atlantic	0.40	0.42	0.42	0.97	0.72	0.73	0.81	0.74	0.79	0.93
East South Central	0.25	0.31	0.26	0.57	0.53	0.47	0.59	0.53	0.61	0.73
West South Central	0.09	0.11	0.12	0.27	0.55	0.24	0.29	0.46	0.34	0.44
Mountain	0.24	0.31	0.31	0.49	0.49	0.49	0.62	0.52	0.46	0.68
Pacific	0.18	0.16	0.17	0.24	0.19	0.26	0.28	0.32	0.48	0.43

Total	**0.40**	**0.41**	**0.45**	**0.74**	**0.70**	**0.75**	**0.91**	**0.86**	**0.99**	**1.08**

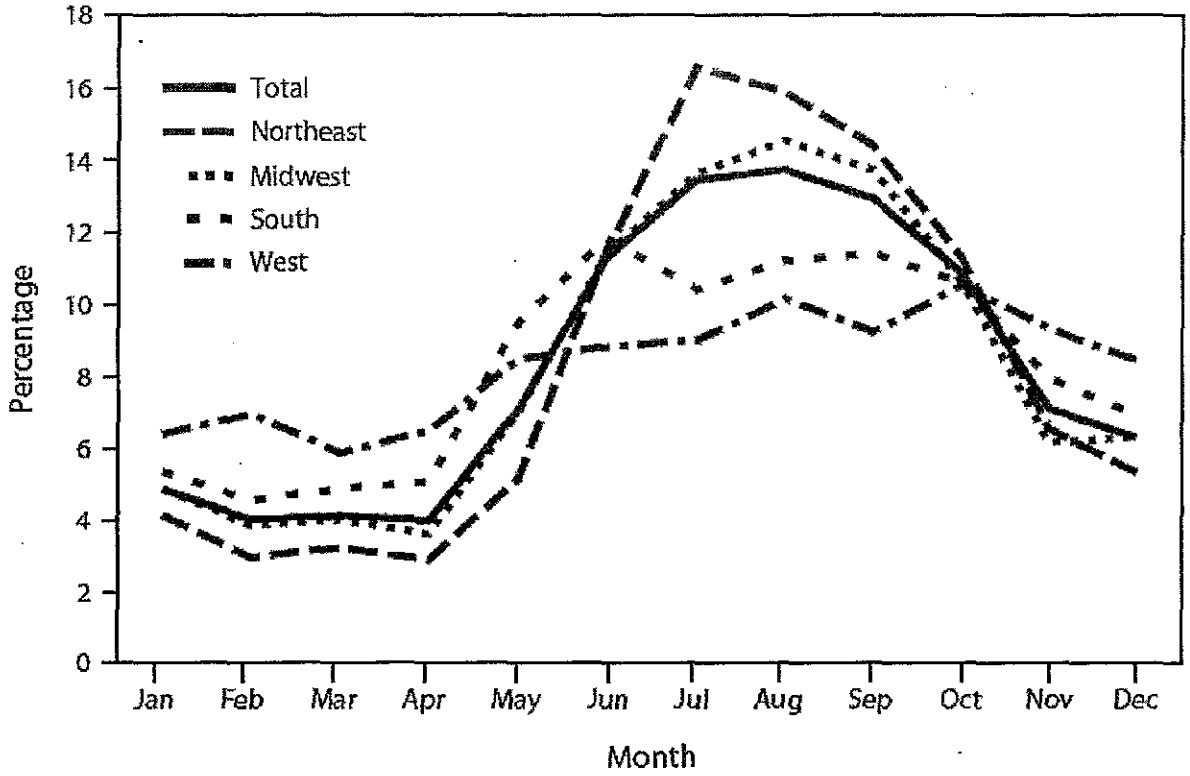
* *New England:* Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont; *Middle Atlantic:* New Jersey, New York, and Pennsylvania; *East North Central:* Illinois, Indiana, Michigan, Wisconsin, and Ohio; *West North Central:* Iowa, Kansas, Missouri, Minnesota, Nebraska, North Dakota, and South Dakota; *South Atlantic:* Delaware, District of Columbia, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia, and West Virginia; *East South Central:* Alabama, Kentucky, Mississippi, and Tennessee; *West South Central:* Arkansas, Louisiana, Oklahoma, and Texas; *Mountain:* Arizona, Colorado, Idaho, Montana, Nevada, New Mexico, Utah, and Wyoming; *Pacific:* Alaska, California, Hawaii, Oregon, and Washington.

TABLE 2. Demographic characteristics of legionellosis cases --- National Notifiable Disease Surveillance System, United States, 2000--2009

Characteristic	No.	(%)	Average per 100,000 population*
Age group (yrs)			
≤9	79	(0)	0.02
10--19	125	(1)	0.03
20--29	516	(2)	0.13
30--39	1,473	(7)	0.36
40--49	3,622	(16)	0.81
50--59	5,401	(24)	1.44
60--69	4,658	(21)	1.94
70--79	3,672	(16)	2.29
≥80	2,864	(13)	2.66
Sex			
Male	14,255	(63)	0.97
Female	8,018	(36)	0.53
Unknown	145	(1)	---
Race			
American Indian/Alaska Native	66	(0)	0.21
Asian	206	(1)	0.14
Black	3,422	(15)	0.87
White	14,287	(64)	0.59
Other	411	(2)	---
Unknown	4,026	(18)	---
Total	**22,418**	**(100)**	**0.75**

* Crude incidence rates, not age-adjusted.

FIGURE. Annual average percentage of legionellosis cases occurring annually, by month and U.S. Census region* --- United States, 2000--2009



* *Northeast:* Connecticut, Maine, Massachusetts, Rhode Island, Vermont, New Jersey, New York, and Pennsylvania; *Midwest:* Indiana, Illinois, Michigan, Ohio, Iowa, Nebraska, Kansas, North Dakota, Minnesota, and Missouri; *South:* Delaware, District of Columbia, Florida, South Carolina, West Virginia, Kentucky, Louisiana, Oklahoma, and Texas; *West:* Colorado, Idaho, New Mexico, Montana, Utah, Nevada, Wyoming, Alaska, California, Hawaii, Oregon, and Washington.

Alternate Text: The figure above shows the average percentage of legionellosis cases occurring in the United States annually, by month and U.S. Census region during 2000-2009. Cases tended to occur in the summer and early fall, with the June-October period accounting for 62% of the cases reported each year.

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医薬品
医薬部外品　　研究報告　調査報告書
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識別番号・報告回数			報告日	第一報入手日 2011 年 8 月 31 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン		研究報告の公表状況	New England Journal of Medicine 2011; 365(5): 422-429	公表国 アメリカ	
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研究報告の概要

エーリキア症(Ehrlichiosis)は、臨床的に重要な新興人獣共通感染症である。Ehrlichia chaffeensis と Ehrlichia ewingii だけが、米国でヒトにエーリキア症を引き起こすと考えられてきた。エーリキア症を疑われる患者は適切な診断を確保し、原因を確かめるために定型的に検査を受ける。我々はエーリキア症の症例の原因を診断し、確かめるために、分子学的方法、培養、及び血清学的検査を用いた。検査では、ミネソタ州とウィスコンシン州のエーリキア症の 4 症例は、Ehrlichia chaffeensis、或いは Ehrlichia ewingii からではない、その替わり新しく発見されたエーリキア種によって引き起こされたであろうことが分かった。全ての患者は発熱、倦怠感、頭痛、及びリンパ球減少症が見られ、3 人は血小板減少症、2 人は肝酵素濃度上昇を有していた。全員ドキシサイクリン治療を受けた後に回復した。
ミネソタ州とウィスコンシン州で採取された 697 匹のクロアシマダニの少なくとも 17 匹は、ポリメラーゼ連鎖反応検査で同じエーリキア種が陽性であった。遺伝子解析は、この新しいエーリキア種が Ehrlichia muris に密接に関連していることを明らかにした。
我々はミネソタ州とウィスコンシン州での新しいエーリキア種を報告し、支援となる臨床的、疫学的、培養、DNA 配列、及び感染源データを提供する。医師は適切な検査、治療、及び地域の監視を確実にするために、この新しく発見された E. muris の近親を知っておく必要がある。

報告企業の意見	今後の対応
アナプラズマ属病原体は、直径0.2～2μmの大きさの球状もしくは楕円状のグラム陰性細菌で、反芻動物、ウマ、ヒトの顆粒球で増殖する。万一、原料血漿にアナプラズマ属病原体が混入したとしても、除菌ろ過等の製造工程にて除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2抗体陰性であることを確認している。更に、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査（NAT）を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した高力価の抗HBs抗体を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗HBs人免疫グロブリンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において60℃、10時間の液状加熱処理及びウイルス除去膜によるろ過処理を施しているが、投与に際しては、次の点に十分注意すること。

ORIGINAL ARTICLE

Emergence of a New Pathogenic Ehrlichia Species, Wisconsin and Minnesota, 2009

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ABSTRACT

BACKGROUND

Ehrlichiosis is a clinically important, emerging zoonosis. Only *Ehrlichia chaffeensis* and *E. ewingii* have been thought to cause ehrlichiosis in humans in the United States. Patients with suspected ehrlichiosis routinely undergo testing to ensure proper diagnosis and to ascertain the cause.

METHODS

We used molecular methods, culturing, and serologic testing to diagnose and ascertain the cause of cases of ehrlichiosis.

RESULTS

On testing, four cases of ehrlichiosis in Minnesota or Wisconsin were found not to be from *E. chaffeensis* or *E. ewingii* and instead to be caused by a newly discovered ehrlichia species. All patients had fever, malaise, headache, and lymphopenia; three had thrombocytopenia; and two had elevated liver-enzyme levels. All recovered after receiving doxycycline treatment. At least 17 of 697 *Ixodes scapularis* ticks collected in Minnesota or Wisconsin were positive for the same ehrlichia species on polymerase-chain-reaction testing. Genetic analyses revealed that this new ehrlichia species is closely related to *E. muris*.

CONCLUSIONS

We report a new ehrlichia species in Minnesota and Wisconsin and provide supportive clinical, epidemiologic, culture, DNA-sequence, and vector data. Physicians need to be aware of this newly discovered close relative of *E. muris* to ensure appropriate testing, treatment, and regional surveillance. (Funded by the National Institutes of Health and the Centers for Disease Control and Prevention.)

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Ehrlichiosis and anaplasmosis are tickborne zoonoses caused by obligate intracellular gram-negative bacteria in the family Anaplasmataceae.[1] Symptoms typically include fever, myalgia, and headache, with rash in rare instances. Severe disease may be associated with gastrointestinal, renal, respiratory, and central nervous system involvement and, in rare cases, death.

In the United States, ehrlichiosis in humans is caused primarily by infection with *Ehrlichia chaffeensis*, which infects monocytes, and less commonly by *E. ewingii*, which infects granulocytes. *Anaplasma phagocytophilum* is closely related to the ehrlichiae and causes human granulocytic anaplasmosis.[1,2] *E. ewingii* and *E. chaffeensis* are transmitted to humans by the bite of an infected tick, *Amblyomma americanum*, whereas *A. phagocytophilum* is transmitted in the United States by the ticks *Ixodes scapularis* and *I. pacificus*.[3]

Ehrlichiosis is a clinically important, emerging zoonosis. *E. chaffeensis*, *A. phagocytophilum*, and *E. ewingii* were first recognized as human pathogens in 1991,[4] 1994,[5] and 1999,[6] respectively. Since then, *E. canis* and *E. muris* have been implicated as causes of human illness in Venezuela and Russia, respectively.[7,8] However, only *E. chaffeensis* and *E. ewingii* have been thought to cause ehrlichiosis in humans in the United States.

METHODS

PATIENTS

EDTA-anticoagulated samples of whole blood obtained from patients throughout the United States with suspected ehrlichiosis or anaplasmosis were submitted for polymerase-chain-reaction (PCR) diagnostic testing for ehrlichia and anaplasma at the Mayo Clinic in Minnesota. Patients with confirmed ehrlichiosis in Minnesota and Wisconsin were interviewed by staff members of local and state health departments according to a standardized questionnaire to obtain demographic, clinical, and epidemiologic information, and medical records were reviewed.

All participants provided written informed consent for collection and testing of additional blood specimens. Research protocols were approved and monitored by the institutional review boards at the Mayo Clinic and the Centers for Disease Control and Prevention (CDC).

REAL-TIME PCR ASSAY

DNA was extracted from the blood specimens (MagNA Pure Instrument, Roche Applied Science) and tested for *E. ewingii*, *E. chaffeensis*, and *A. phagocytophilum* DNA with the use of a real-time PCR assay[9] with primers and fluorescence resonance energy transfer–labeled probes targeting a conserved region of the GroEL heat-shock protein operon. Polymorphisms in the sequence targeted by the probes allowed for differentiation of the three species by means of analysis of melting temperature. Specimens with an atypical result (melting temperature outside the predefined ranges) were tested with the use of a SYBR Green PCR assay targeting the 16S ribosomal RNA gene (*rrs*) of Anaplasmataceae,[10] a nested PCR assay of the GroEL gene (*groEL*),[11] or broad-range *rrs* assays[12] (see Table 1 in the Supplementary Appendix, available with the full text of this article at NEJM.org).

DNA SEQUENCING

Amplified DNA fragments were sequenced (3730 DNA sequencer, Applied Biosystems) and analyzed (Sequencher software, version 4.2; Gene Codes). New sequences were submitted to GenBank (accession numbers HM543745 for *rrs* and HM543746 for *groEL*). New, homologous sequences of infective bacteria and related bacteria were aligned with the use of ClustalW software, and phylogenetic analysis was conducted with the use of Molecular Evolutionary Genetics Analysis software, version 4.0.[13]

CULTURE ISOLATION

Buffy-coat and erythrocyte fractions of the whole-blood specimens were processed and inoculated into a tick cell line (ISE6, derived from *I. scapularis*) and a mammalian cell line (RF/6A, derived from rhesus monkey choroid retina; American Type Culture Collection number CRL-1780), according to published protocols.[14] Mammalian cell cultures were incubated in RPMI 1640 medium with 10% fetal bovine serum at 37°C in 5% carbon dioxide, whereas ISE6 cultures were incubated at 34°C in sealed flasks.[15] Cells were examined microscopically for intracellular morulae (bacterial clusters)

of ehrlichia and anaplasma with the use of phase-contrast or bright-field microscopy.

SEROLOGIC TESTING

Serum and plasma specimens from patients with an atypical *groEL* PCR product were tested for IgG-class antibodies reacting to *E. chaffeensis* or *A. phagocytophilum* with the use of a commercial indirect immunofluorescence assay (Focus Diagnostics).[16] Serum and plasma samples were also tested by means of noncommercial indirect immunofluorescence assays developed and used at the CDC for IgM- and IgG-class antibodies against *E. chaffeensis*, *A. phagocytophilum*, and an ehrlichia species isolated in this study[17]; antigens were derived from canine monocytic DH82 cultures infected with ehrlichia and human promyelocytic HL-60 cultures infected with *A. phagocytophilum*. A reciprocal titer of 64 or higher was considered positive for both assays.

MORPHOLOGIC EXAMINATION OF PERIPHERAL-BLOOD SMEARS

Wright-stained peripheral-blood smears from each patient with an atypical *groEL* PCR product were screened for the presence of intracellular morulae characteristic of ehrlichia species.

TICK COLLECTION AND DNA EXTRACTION

Ticks were collected in June and July 2009 by the Wisconsin Division of Public Health, Medical Entomology Laboratory, University of Wisconsin–Madison and the Minnesota Department of Health. Tick collection was conducted by dragging a fabric flag (1 m by 1 m) across vegetation at or near residences of patients in northwestern Wisconsin and northeastern, central, and northwestern Minnesota. DNA extraction from ticks was performed with the use of a modified version of a published protocol,[18] with three to five nymphs from Wisconsin processed at a time. DNA was tested with the use of the *groEL* fluorescence resonance energy transfer assay and the *rrs* SYBR Green PCR assay.

RESULTS

REAL-TIME PCR ASSAY AND SEQUENCING

From June 1 through December 31, 2009, a total of 4247 blood specimens from residents in 45 states were tested by means of *groEL* PCR assay. Of the 1518 specimens obtained from Wisconsin and Minnesota residents, 163 (10.7%) were positive for *A. phagocytophilum* (35 from Wisconsin and 128 from Minnesota), whereas none were positive for *E. chaffeensis* or *E. ewingii*. Three additional Wisconsin residents and one Minnesota resident had positive PCR tests with a melting temperature that was outside the melting temperature range for *E. chaffeensis*, *E. ewingii*, and *A. phagocytophilum* (Fig. 1 in the Supplementary Appendix). This atypical result was not found for the 2729 specimens collected from the 43 other states.

The four specimens with an atypical *groEL* PCR melting temperature also tested positive for Anaplasmataceae *rrs* with the use of the SYBR Green PCR assay. The nucleotide sequences of the amplified *rrs* and *groEL* fragments were identical among the four specimens and shared 98% sequence similarity with the homologous *rrs* and *groEL* genes of *E. muris* (Fig. 1).

CULTURE ISOLATION

Two ehrlichia species isolates (designated Wisconsin 1 and 2) were cultured from blood specimens obtained from one of the four patients 3 and 4 days before culturing in ISE6 and RF/6A cell lines. Sequence analysis of the PCR-amplified portions of *rrs* showed that they were identical to each other and to the sequences obtained from the clinical specimens with the atypical melting-temperature results.

Morulae were detected with the use of phase-contrast microscopy of live RF/6A cultures 5 weeks after inoculation. Fixed and stained ISE6 cells contained one to three large morulae per cell, whereas RF/6A cells contained numerous, small morulae (Fig. 2).

SEROLOGIC TESTING

Of the four patients with atypical PCR results, two (Patients 2 and 4) (Table 1) were tested by means of the commercial indirect immunofluorescence assay. Serum samples collected from Patient 2 were negative for IgG antibodies to *E. chaffeensis* (i.e., titer <64) on days 2 and 15 after the onset of fever, whereas serum specimens from Patient 4 were positive (i.e., titer ≥64) for IgG antibodies on day 5 (titer of 256) and day 54 (titer of 1024) after the onset of fever.

In addition, serum and plasma specimens from three of these four patients were tested by means of the CDC indirect immunofluorescence assays. At least one specimen from each patient tested was positive for IgM or IgG antibodies reacting to *E. chaffeensis*, and the titers were even higher in response to the new ehrlichia species. A speci-

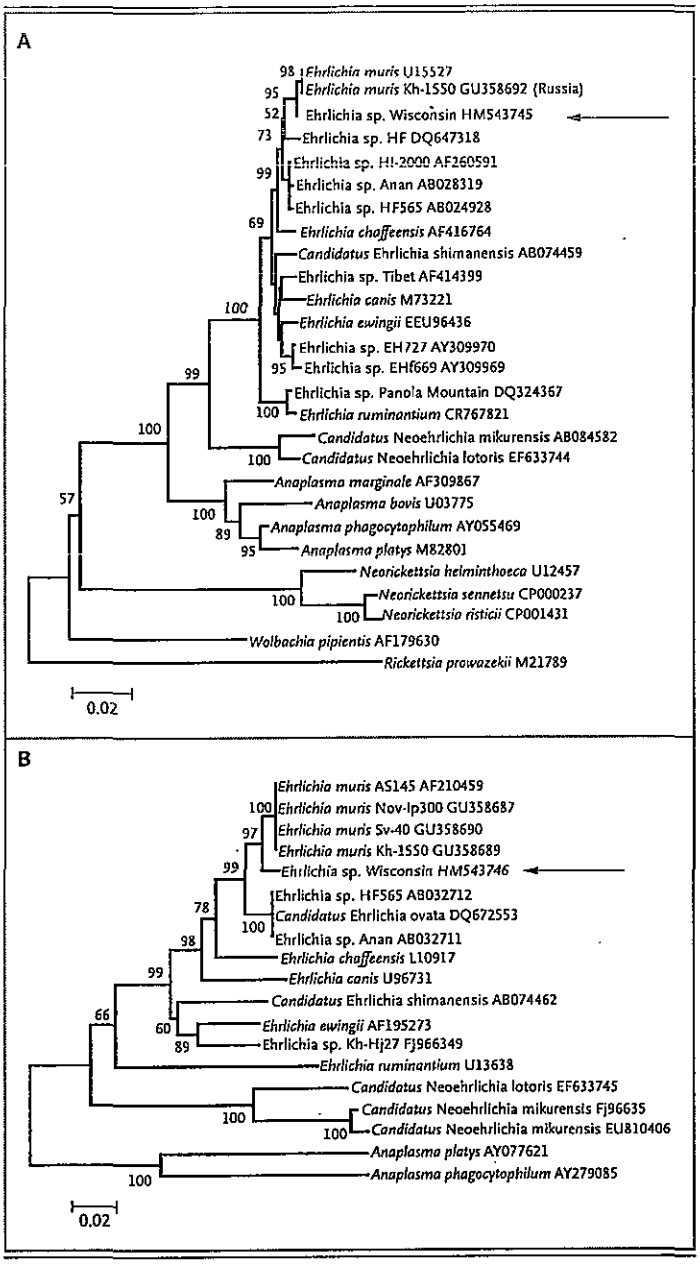

Figure 1. Genetic Relationships between the New Ehrlichia Species and Related Bacteria.

The arrow to the right of each phylogenetic tree indicates the newly discovered ehrlichia species (called "Wisconsin"). Panel A shows the phylogeny based on the 16S ribosomal RNA gene (*rrs*), inferred with the use of the minimum-evolution method and with distances calculated by means of the Jukes–Cantor method as the number of base substitutions per site. Panel B shows the phylogeny based on the GroEL heat-shock protein operon gene (*groEL*), inferred with the use of the neighbor-joining method and with distances calculated by means of the Kimura two-parameter method as the number of base substitutions per site. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (of 1000 replicates) is shown to the left of each branch. The trees are drawn to scale, with branch lengths in the same units as those of the evolutionary distances (see scale bars) used to infer the phylogenetic tree. Positions containing gaps, missing data, and primer sequences were eliminated from the data set. A total of 1160 positions for *rrs* and 591 positions for *groEL* were analyzed. Phylogenetic analyses were conducted with Molecular Evolutionary Genetics Analysis software, version 4.0.[13] The GenBank accession number is listed at the end of each isolate name.

men obtained 15 days after the onset of illness from Patient 2 had high titers of IgM and IgG antibodies against the new ehrlichia species; in a specimen obtained 188 days after onset, IgM and IgG antibody titers were substantially reduced. Patient 4 had a strong seroconversion with a high IgG antibody titer 76 days after infection. No antibodies reacting to *A. phagocytophilum* antigens were detected (i.e., titer <64) in three patients with the use of the commercial or noncommercial assay. Specimens from Patient 3 were not available.

PATIENTS AND CLINICAL PRESENTATION

The four patients had an onset of illness between June 8 and October 27, 2009. Their ages ranged from 23 to 51 years; two were men (Table 2). All four patients whose specimens were positive for the newly discovered ehrlichia species reported fever, fatigue, and headache. Patient 2 also reported nausea and vomiting. The interval between the onset of illness and the physician visit was 1 to 4 days. Laboratory findings included lymphopenia (in all four patients), thrombocytopenia (in three), elevated hepatic aminotransferase levels (in one of the three patients tested), and mildly elevated alkaline phosphatase levels (in one of the two patients tested). No morulae or other blood parasites were detected in peripheral-blood smears.

Two patients had previously received solid-organ transplants and were taking immunosuppressive drugs at the time of diagnosis. One patient had cystic fibrosis and had undergone bilateral lung transplantation 2 years before the onset of illness; medications received included mycophenolate mofetil, cyclosporine, and prednisone. This patient was hospitalized for 3 days during the acute illness for management of an infiltrate in the left lung and pleural effusion on the left side for which a specific cause was not

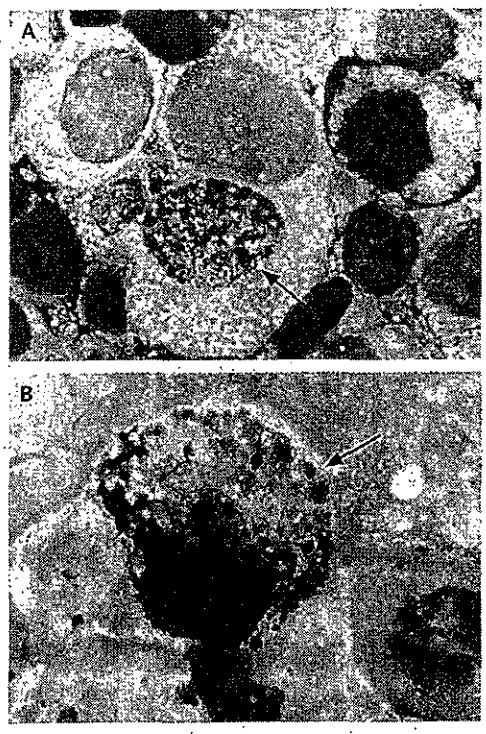

Figure 2. Intracellular Morulae of the New Ehrlichia Species in the ISE6 and RF/6A Cell Cultures.
Panel A shows the ISE6 cell line, and Panel B shows the RF/6A cell line. Morulae are indicated by arrows (Giemsa stain).

determined. The symptoms improved after administration of ceftazidime and doxycycline. The second patient had received a renal allograft 4 years before the onset of symptoms and was receiving mycophenolate mofetil, tacrolimus, and prednisone. This patient had acute kidney injury (serum creatinine level, 2.2 mg per deciliter [194.5 μmol per liter] vs. a baseline of 1.2 mg per deciliter [106.1 μmol per liter]) and was successfully treated with doxycycline. The two immunocompetent patients had relatively mild illnesses and were successfully treated with doxycycline. The patient from Wisconsin received doxycycline at 100 mg twice daily for 21 days, and the patient from Minnesota received the same regimen for 10 days.

EPIDEMIOLOGIC INVESTIGATION

All patients reported peridomestic (e.g., from mowing the lawn) or recreational exposure to ticks or wooded areas (Fig. 2 in the Supplementary Appendix). The three Wisconsin patients resided in Eau Claire or Burnett County, and one reported traveling to Bayfield County in northwest Wisconsin 1 week before the onset of illness. The Minnesota patient resided in Rice County and had traveled to a wooded area in Pine County, Minnesota, within 30 days before the onset of symptoms.

TICK COLLECTION AND PCR ASSAY

A total of 697 ticks were tested. DNA from the newly discovered ehrlichia species was detected in 16 of 534 *I. scapularis* ticks (7 nymphs and 9 adults) from Minnesota, as well as in 1 group of 5 nymphs (of 154 total) from Wisconsin (where the minimum infective rate is 6.5 infected nymphs per 1000 nymphs tested) (Table 2 in the Supplementary Appendix). No DNA from the newly discovered ehrlichia species was detected in 9 *I. scapularis* adults from Wisconsin or 88 *Dermacentor variabilis* adults from Wisconsin.

DISCUSSION

We have identified a new ehrlichia species (subsequently referred to as ehrlichia species Wisconsin) in blood from four patients living in Wisconsin or Minnesota, by using molecular, culture, and serologic methods. The presence of ehrlichia species Wisconsin DNA in blood specimens from these patients collected during the period of acute illness suggests that this organism was the etiologic agent of their infection. This is supported by the results of serologic testing with whole-cell antigens of the Wisconsin isolate: IgM and IgG antibody responses against the species were positive in the three patients tested, with consistently higher titers than those to *E. chaffeensis*. All four patients recovered after administration of doxycycline, which is the antibiotic of choice for the treatment of ehrlichiosis.

The identification of ehrlichia species Wisconsin in humans has important clinical and epidemiologic implications. Ehrlichiosis was not previously thought to be endemic in Minnesota and Wisconsin and would not be routinely tested for among patients from these areas. Also, commercial tests for ehrlichiosis may fail to provide an accurate identification of this organism. The considerable serologic cross-reactivity of the Wisconsin isolate with *E. chaffeensis* could confound diagnostic and epidemiologic studies and may explain the recent increase in the numbers of cases attributed to *E. chaffeensis* infection in Wisconsin and Minnesota on the basis of serologic testing

Table 1. Results of Tests for IgM and IgG Antibodies against the New Ehrlichia Species, *Ehrlichia chaffeensis*, and *Anaplasma phagocytophilum*.*

Patient No.†	Days from Illness Onset to Specimen Collection	Specimen Type	Reciprocal IgM and IgG Titer, CDC Assay						Reciprocal IgG Titer, Mayo Clinic Assay	
			New Ehrlichia Species		*E. chaffeensis*		*A. phagocytophilum*		*E. chaffeensis*	*A. phagocytophilum*
			IgM	IgG	IgM	IgG	IgM	IgG		
1	198	Plasma	256	128	256	<16	<16	<16	NA	NA
2	2	Serum	NA	NA	NA	<32	NA	<32	<64	<64
2	15	Serum	512	256	256	64	<16	<16	<64	<64
2	188	Plasma	16	64	<16	32	<16	<16	NA	NA
4	7	Serum	NA	NA	NA	NA	NA	NA	256	<64
4	46	Serum	NA	NA	NA	NA	NA	NA	1024	<64
4	76	Plasma	32	2048	<16	2048	<16	<16	NA	NA

* Antibody titers were assessed with the use of a noncommercial indirect immunofluorescence assay at the Centers for Disease Control and Prevention and with the use of a commercial indirect immunofluorescence assay (testing for IgG antibody but not IgM antibody) at the Mayo Clinic. NA denotes not available.

† Specimens from Patient 3 were not available for testing.

only. In addition, PCR assays for *E. chaffeensis* and *E. ewingii* may not detect ehrlichia species Wisconsin because of lack of primer and probe homology. The ehrlichia–anaplasma real-time *gro*EL PCR assay used in our investigation has the advantage of providing differential detection of ehrlichia species Wisconsin from *E. chaffeensis*, *E. ewingii*, and *A. phagocytophilum* on the basis of differences in DNA composition of the amplified fragment.[9] Finally, detection of morulae in peripheral-blood samples from infected persons is an unreliable means of diagnosing infection with ehrlichia species Wisconsin. Morulae are detected infrequently in blood from patients infected with ehrlichia species[19] and were not found in blood from our four patients.

Ehrlichia infections in the United States are commonly transmitted by *A. americanum*. However, the northern range for *A. americanum* is not thought to extend into Wisconsin and Minnesota, and public submissions of *A. americanum* ticks to the University of Wisconsin–Madison or the Minnesota Department of Health are uncommon. In contrast, both *D. variabilis* and *I. scapularis* are abundant, human-biting species in northwestern Wisconsin and Minnesota. The presence of ehrlichia species Wisconsin DNA in at least 17 *I. scapularis* nymphs and adults, as well as the absence of ehrlichia DNA in the *D. variabilis* ticks tested, suggests that *I. scapularis* is a vector for ehrlichia species Wisconsin. Extended investigation and tick surveillance are required to understand the distribution of this agent in Wisconsin and Minnesota and to definitively implicate a specific tick vector.

The new ehrlichia species reported in this study is closely related to *E. muris* (with approximately 98% sequence homology), but its exact taxonomic placement cannot yet be determined, because only a few isolates and limited genetic data are available. *E. muris* is considered to be an Old World pathogen found in different ticks of the *I. persulcatus* complex ranging from Eastern Europe to Japan.[10,20,21] *E. muris* DNA has been detected in the blood of small rodents and deer from these areas,[22] suggesting that these animals may be reservoirs of *E. muris* and related organisms. We are also aware of at least 2 PCR-confirmed and 84 serologically diagnosed cases in humans attributed to *E. muris* infection in the Perm region of Russia.[12] Similarly, Japanese investigators reported a 1.1% seroprevalence of antibodies against *E. muris* among 1893 Tokyo residents, with an even higher seroprevalence among rodents (6 to 63%)[23]; however, it is difficult to ascertain whether these antibodies in mice and humans are related to *E. muris* or to other antigenically related organisms, because multiple ehrlichia agents have been reported from the same region.[10,21,24]

In summary, we have characterized a newly discovered ehrlichia species with supportive clini-

Table 2. Laboratory Test Results in the Four Patients Infected with the New Ehrlichia Species, Shortly after Presentation.*

Patient No.	Age	Sex	White-Cell Count	Lymphocyte Count	Platelet Count	AST	ALT	Alkaline Phosphatase
	yr			$\times 10^{-9}$/liter			U/liter	
1	51	M	3.4	0.48	87	76	75	NA
2	23	M	3.6	0.41	104	42	NA	134
3	50	F	5.0	0.84	132	NA	NA	NA
4	50	F	3.6	0.54	212	16	NA	88
Normal range			3.5–10.5	0.9–2.9	150–450	8–48	7–55	45–115

* ALT denotes alanine aminotransferase, AST aspartate aminotransferase, and NA not available (not performed or not reported).

cal, epidemiologic, culture, DNA-sequence, and vector data. Further assessment of the ecologic, epidemiologic, and clinical features of the infection caused by this species is required to facilitate its distinction from other known tickborne infections in this region. To guide diagnostic testing and treatment, physicians should be aware that a novel pathogenic ehrlichia agent is present in Minnesota and Wisconsin and that organism-specific PCR and serologic testing can be used to identify the cause of suspected infections.

Note added in proof: After this article was submitted, Telford et al. reported findings of an *E. muris*–like bacterium in Wisconsin *I. scapularis* ticks collected during the 1990s.[25]

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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別紙様式第2-1

No. 20

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 6. 23	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況		公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)		Levicnik-Stezinar S. 21st Regional Congress of the International society of Blood Transfusion, June 18-22, 2011, Lisbon, Portugal.	スロベニア

研究報告の概要

○ヒト顆粒球エーリキア症の輸血感染
ヒト顆粒球アナプラズマ症(HGA;以前はヒト顆粒球エーリキア症として知られていた)は、アナプラズマ・ファゴサイトフィルムによるダニ媒介性の人畜共通感染症である。赤血球輸血によるアナプラズマ感染の可能性について報告する。
36歳女性が帝王切開術を受け、その際に6単位の赤血球と2単位の新鮮凍結血漿が輸血された。9日後に発熱を生じ、後に急性呼吸窮迫症候群(ARDS)に転帰した。PCRによってアナプラズマ・ファゴサイトフィルムが検出され、HGAであることが確認された。感染原因として可能性のあるものは輸血のみであった。輸血された8単位についてPCR及び間接蛍光抗体法により検査を行った結果、1検体が陽性を示した。
今回、ARDSを伴う輸血感染HGAの重篤症例を確定した。感染は白血球除去赤血球に起因したため、白血球除去の効果は小さいと思われる。この感染症例は、ダニ咬傷歴のある供血者の一時的な供血停止を支持する。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
急性呼吸窮迫症候群を伴う重篤な輸血感染ヒト顆粒球エーリキア症が発生したとの報告である。	今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

20

2009 (none WNV positive) and 14,786 in 2010 (2. 1.3% WNV positive). For both the considered methods the need of test repetition was low (under 3%).
Discussion: WNV is a relatively new health threat in Italy and his impact on the National Blood systems is not predictable. Thus, the most effective strategies: surveillance, donors deferral, NAT screening will have to be re-evaluated over time. The rapid introduction of the WNV NAT assay in response to the notification, in 2009, of human neuro-invasive NWV infections is an example of the ability of regulatory agencies, blood testing organizations, and test manufacturers to respond quickly to emerging blood-borne pathogens.

P-384
TRANSFUSION TRANSMITTED HUMAN GRANULOCYTIC EHRLICHIOSIS
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Background: Human granulocytic anaplasmosis (HGA; previously known as "human granulocytic ehrlichiosis") is a tick-born zoonosis that is caused by an obligate intracellular bacterium, Anaplasma phagocytophilium. Human infection is usually tick borne.
Aims: A possible transmission of anaplasma acquired through a transfusion of red blood cells is reported.
Case report: An elective caesarean section and consecutive surgical revision was performed on a 36-year-old woman. 6 units of RBC and 2 units of FFP were transfused. 9 days later the patient developed a fever, which in few days turned into acute respiratory distress syndrome (ARDS). An infection with HGA was confirmed. The only possible cause of infection could have been in the transfusion of blood components.
Methods: The diagnosis of the patient was confirmed when Anaplasma phagocytophilum was detected using PCR. The PCR and IFA serology testing for IgG Anaplasma phagocytophilum was performed on the 8 transfused donations.
Results: One of the blood donor samples was retrospectively identified as PCR positive and IgG reactive (>1:1024)
Table 1: Results of testing donor and recipient for anaplasma phagocytophilum

RECIPIENT			DONOR		
	PCR	IFA		PCR	IFA
			-118 days	-	-
Day of transfusion	-	-	Day of donation (8 days before transfusion)	+	>1 : 1.024
+ 15 days	+	-	+ 43 days	-	>1 : 2.048
+ 19 days	+	+			
+ 25 days	-	+			

Conclusions: We investigated and confirmed the case of severe HGA with ARDS transmitted by blood transfusion. The infection occurred after the transfusion of leucoreduced red blood cells. The efficacy of leucoreduction is probably small. There were no other recipients of the disputable donation. A psoralen inactivated unit of pooled platelets had already expired and was not used for transfusion. The FFP was destroyed. This proven transmission supports the indication for the temporary deferral of blood donors with a history of tick bites.

P-385
SEN VIRUS INFECTION IN IRANIAN THALASSEMIC PATIENTS
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Background and objectives: SEN virus (SEN-V) is a blood-borne, single-stranded, non enveloped DNA virus.
Beta-thalassemia is very common in Iran and some Mediterranean countries. Most of these patients need blood transfusion which increases the risk for blood -borne viruses. The aim of this study was to investigate the prevalence of SEN-V-D and-H viremia among thalassemic patients.
Material and methods: Sera of 260 blood donors and 40 thalassemic patients,who were negative for serum Hepatitis B surface antigen (HBsAg) and third-generation HCV antibody (anti-HCV) were tested for SENV-D and -H DNA. DNA was extracted from plasma samples and amplified by seminested PCR.
Results: SENV-D viremia was detected in 4 (1.54%) and 2 (5%) of 260 blood donors and 40 thalassemic patients respectively. SENV-H virenia was detected in 47 (18.08%) and 18 (45%) of 260 blood donors and 40 thalassemic patients respectively. SENV-D or SENV-H virenia was identified in 60 (23.08%) and 19 (47.5%) of 260 blood donors and 40 thalassemic patients respectively.
Conclusions: The prevalence of SENV-H is more than SENV-D. SENV infection is higher in thalassemic Hepatitis than in blood donors. The high frequency of SENV infection among thalassemic patients could be associated with blood transfusion
Key Words: Blood donor, Prevalence, SEN Virus, Thalassemic patient.

P-386
EFFICACY OF TEMPORARY EXCLUSION OF TRAVELLING BLOOD DONORS
Prinsze F, Zaaijer HI.
Sanquin Research, Amsterdam, The Netherlands

Background: Travelling donors who unknowingly import exotic infections endanger the safety of the blood supply. Outbreaks should be evaluated rapidly after the first signals of an emerging disease appear; only a limited time frame is available for analysis, decision making and implementation of safety measures. Using deterministic calculations we estimated in hindsight the threat of five emerging epidemics abroad, that did (n = 3) and did not cause (n = 2) temporary deferral of travelling Dutch blood donors.
Method: Three outbreaks were analysed that caused temporary exclusion of donors returning from the area involved: (1) an outbreak of West Nile Virus (WNV) in Emilia Romana-Italy (78 notified cases with hospitalizations); (2) WNV outbreak in West Macedonia-Greece (108 notified cases); and (3) the Chikungunya outbreak in Thailand (12115 notified cases). Two outbreaks without deferral of returning donors were analysed: (4) the Sandfly Fever Sicilian Virus (SFSV) outbreak in the Ankara region-Turkey (260 notified cases) and (5) the Hepatitis A outbreak in Latvia (2817 notified cases).
Sources of data and assumptions:
(1) Data on Dutch tourists were obtained from the Dutch Bureau of Statistics. We assume that 5% of Dutch tourists are blood donors. Because donors are relatively healthy they are assumed to travel twice as much as the general population. (2) Dutch tourists are assumed to visit the same regions as all tourists; as reported by statistical offices in the countries involved. (3) The incidence among tourists (i.e. per time unit) is assumed to equal the reported incidence in the local populations.
Results: The estimated numbers of returning, infected Dutch blood donors are as follows: (1) WNV outbreak in Emilia Romana resp. in Macedonia: 0.2 resp. 0.5 infected returning donors.
(2) Chikungunya outbreak in Thailand: 1.6 infected returning donors.
(3) Sandfly virus fever in Ankara region: 0.06 infected returning donors.
(4) Hepatitis A in Latvia: 0.6 infected returning donors.

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2011. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況		公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)		IASR Vol.32 No.8 (No.378) August 2011; Available from: http://idsc.nih.go.jp/iasr/32/378/dj3781.html	日本

研究報告の概要

○北海道のライム病の発生状況と症例－自験113例の検討

ライム病は起因菌であるスピロヘータの一種*Borrelia burgdorferi* sensu lato(ボレリア)を保有するマダニ類(日本ではシュルツェマダニ)の媒介により生じる全身性感染症である。臨床症状は早期(I、II期)、後期(III期)に大別され、早期は慢性遊走性紅斑(ECM)、顔面神経麻痺などの神経症状、関節痛などをきたし、後期には慢性萎縮性肢端皮膚炎(ACA)、関節炎などを呈する。日本におけるライム病は1987年に長野で1例目が報告されて以来、主に北海道、本州中部以北で200例以上の確定例の存在が推定される。ライム病がマダニ刺咬症のうちどのくらいの頻度で発症するかは不明であったが、1995年～2000年の6年間に北海道の道北道東地方の関連病院を中心に集積したマダニ刺咬症700例のうち確定例が56例(8.0%)であることから、ボレリア汚染地域においてライム病が発症する頻度はマダニ刺咬症の10%未満と推定される。また1989年から2004年までに113例の確定例を集積し、そのうち52例は皮疹部からのボレリア分離培養に日本で初めて成功した。北海道のライム病はECMに代表される皮膚症状が主体で、第II期以後の出現頻度も9例(8.0%)と欧米に比べ低い。また発熱、全身倦怠感などの全身症状の出現頻度もそれぞれ29例(26%)、11例(9.7%)と低く、抗菌薬に対する反応も良好で、一般に軽症例が多い。北海道に代表される日本のライム病が概して軽症である原因は、ボレリアそのものの病原性の違いや、人種的遺伝的違い、抗菌薬を早期に使用できる医療状況、vectorであるマダニの違いなど、複数の要因が関与していると推定される。

報告企業の意見	今後の対応
北海道を中心とした日本国内のライム病症例を検討することにより、日本におけるライム病の特徴を示した報告である。	今後も引き続き情報の収集に努める。

使用上の注意記載状況・その他参考事項等

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

12

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北海道のライム病の発生状況と症例—自験113例の検討

(Vol. 32 p. 218-219: 2011年8月号)

1. はじめに

ライム病は起因菌であるスピロヘータの一種の*Borrelia burgdorferi* sensu latoを保有するマダニ類(日本ではシュルツェマダニ)の媒介により生じる全身性感染症である。臨床症状は早期(I、II期)、後期(III期)に大別され、早期は慢性遊走性紅斑(erythema chronicum migrans; ECM)、顔面神経麻痺などの神経症状、関節痛などをきたし、後期には慢性萎縮性肢端皮膚炎(acrodermatitis chronica atrophicans; ACA)、関節炎などを呈する。特徴的なECMがみられれば比較的容易に診断可能だが、マダニ刺咬の既往が不明で、関節、神経症状主体の症例は診断が困難で、血清診断や、病変部の培養などの検査を必要とする。筆者は2004年までに旭川医大皮膚科で113例のライム病を経験し1)、これは本邦の確実例の半数以上を占めると推定される。筆者が現在の病院へ赴任後も毎年3～4人のライム病患者が来院しており、北海道に確実例が年間10例は発生すると推定している。したがって2010年までに、北海道のライム病確実例は200例を超えると推定する。ここで自験113例のライム病の臨床的特徴を概説し、ライム病の最近の症例も含めて紹介したい。

2. ライム病の一般的臨床所見と自験ライム病のECM

ライム病の症状は早期(I、II期)、後期(III期)に大別され、以下に概説する。

I期(局在期):ECMはマダニ刺咬部から紅斑性丘疹で始まり、周辺に紅斑が拡大する。易疲労感、発熱、筋肉痛、頚部痛などの症状をともなったり、関節痛、リンパ節腫脹もみられることがあり、約4週間続く。

II期(播種期):ECMが多発性にみられたり(二次性遊走性紅斑)、皮膚リンパ球腫、循環器症状としてA-V blockや心膜炎などが稀にみられる。また、顔面神経麻痺、神経根炎、髄膜炎などもみられ、数週間～数カ月続く。

III期(慢性期):数カ月～数年にわたり、ACA、慢性の髄膜炎、視神経萎縮、大関節の腫脹と疼痛を伴った慢性関節炎がみられる。

これらI～III期の症状が順番に出現せずに、いきなりII期の症状(顔面神経麻痺)が発症することもあるが、後に詳細な病歴をとるとECMの存在していたことが発覚することもある。欧米でもECMの出現頻度は当初は50～70%といわれていたが、患者教育とライム病の診断を厳格化することで、その頻度は90%に達するといわれている。また、自験例では後に述べるようにECM主体の皮膚症状(I期)でとどまる症例が多い。以下にECMの臨床像を述べる。

ECMはマダニ刺咬後、数日～1カ月後に刺咬部を中心に丘疹状紅斑で始まり、急速に拡大して環状になり、径10cm以上になる。典型的なものでは「bull's eye」と表現されるring-shaped erythema(環状紅斑)が多く、homogeneous erythema(均一紅斑)も頻度が高い。稀に紅斑が紫斑状になり、hemorrhagic erythema(出血性紅斑)になることもある。稀に浮腫性紅斑で、小水疱、膿疱を伴い蜂窩織炎様2)の臨床像も呈する。さらに径1～2cm程度のatypical stationary erythemaも稀に存在する。ふつう自覚症状は強くないが、掻痒感、灼熱感を伴う。自験113例では環状紅斑が72例(64%)、均一紅斑が36例(32%)であった。ダニ刺咬部は多くは硬結、時に壊死、痂皮を伴う。皮膚外症状としてはECMに伴って発熱、全身倦怠感、頚部痛、筋肉痛、ダニ刺咬部の近くの関節痛、リンパ節腫脹がみられる。本邦では5%以下に顔面神経麻痺などの神経症状がみられると推定される。

3. 自験ライム病113例の特徴

本邦では1987年に長野で1例目が報告3)されて以来、主に北海道、本州中部以北で200例以上の確実例の存在が推定される。発症地域が限られる理由は、起因菌ボレリア(*B. burgdorferi* sensu lato)を保有するマダニが現時点ではシュルツェマダニに限られ、このマダニは本州では標高1,000m以上の山岳地帯および、北海道などの寒冷地域に生息するためと推定される。シュルツェマダニのすべてが*B. burgdorferi* sensu latoを有しているわけではなく、15～22%の保有率である。本邦症例はマダニ刺咬の既往を患者が記憶していることが多いが、欧米ではマダニ刺咬の既往歴は1/3程度である。これは欧米ではサイズの小さな若虫による刺咬が多く、患者が気づかないのに対して、わが国のシュルツェマダニの刺咬はほとんどが成虫によるため、吸血によりかなり大きなサイズになり、患者が認識しやすいためと推定される。

ライム病がマダニ刺咬症のうちどのくらいの頻度で発症するかは不明であったが、1995～2000年の6年間に北海道の道北道東地方の関連病院を中心に我々が集積したマダニ刺咬症4)は700例あり、そのうちECMが発症し、ボレリア培養陽性あるいは血清抗体陽性のライム病確実例が56例(8.0%)であることから、ボレリア汚染地域においてもライム病が発症する頻度はマダニ刺咬症の10%未満と推定される。我々は1989年に1例目5)のライム病を報告して以来、前述したごとく2004年までに113例の確実例を集積し、そのうち52例はBSKII

培地を用いて、皮疹部からのボレリア分離培養6,7) に本邦で初めて成功した。

本症の生命予後は良好であり、北海道のライム病はECMに代表される皮膚症状が主体で、第II期以後の出現頻度も9例(8.0%)と、欧米に比べ低い。また、発熱、全身倦怠感などの全身症状の出現頻度もそれぞれ29例(26%)、11例(9.7%)と低く、抗菌薬に対する反応も良好で、一般に軽症例が多い。また、欧米の第III期にみられるような慢性のリウマチ様関節炎を呈した症例はなく、一過性の関節痛が22例(19%)に認められた。これらの関節痛は治療に対する反応もよく、ECMの消褪とともに症状が消失する。ただし、1999年に胸鎖関節炎の合併を整形外科医によって診断されたIII期の確実例も道東で発生した。顔面神経麻痺が3例8-10) (2.7%)にみられ、髄膜炎9) も認められた。また稀ではあるが、治療に伴うJarisch-Herxheimer反応11) が生じることも留意すべきである。また、最近ではかなりの肝障害がみられた症例もあり、この症例では担当の内科医もライム病関連の肝炎を疑っている(本号6ページ参照)。

北海道に代表される本邦のライム病が概して軽症である原因は、ボレリアそのものの病原性の違いや、人種的遺伝的違い、抗菌薬を早期に使用する医療状況、vectorであるマダニの違いなど、複数の要因が関与していると推定される。他方、世界的にみると、慢性期ライム病では、抗菌薬による治療後も年余にわたって、倦怠感、全身の筋肉痛、知覚異常、言語の記憶力低下などの神経症状が継続することが知られ、急性期症状から引き続き生じる鬱症状との鑑別が以前から問題になっていた。これについて最近は症例の集積がなされて、meta-analysis の結果12) 、2006年のISDA(Infectious Disease Society of America)のガイドラインではpost-Lyme disease syndrome (PLDS)が提唱されている。PLDSの治療については抗菌薬の追加投与が有効か否かのcontrolled trialがなされて、プラセボと有意差がない結果となった。したがって、このような症例を経験した場合は、適切な抗菌薬による治療を1コースのみ追加して行い(エビデンスがないことを念頭に入れて)、その後は対症療法(たとえばアミトリプチリン・商品名トリプタノール)などが推奨されている。我々は適切な治療後も軽度の倦怠感、マダニ刺咬部位近くの神経知覚障害、関節痛が持続するPLDSと思われる症例を1例経験したので報告する。

4. 症例報告

症例:41歳、男性
初診:2006年10月20日
主訴:左下肢のしびれ、索状硬結、倦怠感、眩暈、動悸

現病歴:2006年6月8日、上ノ国町の山で左下腿をマダニ刺症。自分で抜去した。9月中旬から同部位に浸潤性紅斑出現し、拡大するため札幌医大皮膚科初診し、血清ウエスタンブロットにて*B. garinii* 抗体がIgG、IgMともに陽性でライム病と診断され、ミノサイクリン投与をうけた。紅斑は消退したが、主訴の訴えが残り、当科を初診した。2週間テトラサイクリンの内服でも軽快せず、集中力低下、倦怠感が強く、入院治療を希望したため、2006年11月9日当科に入院治療となった。

現症:左下肢に淡褐色の約1cmの硬結(マダニ刺咬部)とその上方に静脈に沿って淡い紅斑が認められた。

入院時検査所見:血液生化学所見はWBC 5,700、Hb 13.7、PLT 23.5万、CRP 0.37、RF 3.4、ASO 18、ALP 263 、CH50 53.1、AST 18、ALT 10、LDH 171、BUN 11.9、CRE 0.94、CPK 185と異常なく、ボレリア抗体0.42(EIA)、心電図、心エコー異常なし、HLA検索はHLA A2、A33、B61、B44、DR9、DR13。

臨床経過:PLDSまたは慢性期ライム病を考え、セフトリアキソン2g／日の点滴を4週間継続した。下肢の索状硬結は改善し、動悸もみられなくなった。しかし、倦怠感と下肢の鈍痛が持続した。退院後はEBM がないことを説明の上、ドキシサイクリン内服を開始した。1カ月後から倦怠感、下肢の鈍痛、集中力低下も改善。本人の希望もあり、さらに1カ月内服して治療中止した(2007.3.16)。仕事への意欲も出て、4月から復帰するという。その後、2カ月に1回経過を見せにくるが、元気である(2008.1.18終診)。経過を通じてアミトリプチリンは使用しなかった。

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JA旭川厚生病院皮膚科　橋本喜夫

今月の表紙へ戻る

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IASR *Infectious Agents Surveillance Report*

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医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 9. 15	該当なし	

一般的名称	解凍人赤血球濃厚液	研究報告の公表状況	CDC Media Relations, September 6, 2011; Available from: http://www.cdc.gov/media/releases/2011/p0906_parasitic_infection.html?s_cid=2011_p0906_parasitic_infection.html	公表国
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)			米国

研究報告の概要

○ダニ媒介の寄生虫感染に対する米国における血液供給の脆弱性 リスク軽減のためには供血者スクリーニングのバベシア検査が必要

米国疾病管理予防センター(CDC)と共同研究者が行った過去30年にわたる研究の結果によると、バベシアは赤血球内のダニ媒介性寄生虫であり、輸血によって感染しうる。輸血関連バベシア症は初めて発生した1979年以来次第に認識されてきた。感染しているにも関わらず自覚のない供血候補者に対してFDA認可のバベシア検査は万能ではない。バベシア症は生命に関わるが治療可能な疾患であるため、スクリーニング検査の向上を含めて予防の戦略が必要である。複数のメーカーが血液事業者とともにバベシア検査の開発に取り組んでいる。

バベシア症は通常ダニによって媒介されるため、ダニに刺咬され無意識のうちに感染した人からの輸血で伝播することもある。従って、ダニ媒介感染の予防が血液供給の安全対策を助けることとなる。

米国のほとんどのダニ媒介バベシア症は7つの州で(コネチカット州、マサチューセッツ州、ミネソタ州、ニュージャージー州、ニューヨーク州、ロードアイランド州、ウィスコンシン州)、特に暖かい時期に発生している。しかし輸血関連バベシア症は19州において認識されており、年間を通して発生している。

バベシア症はマラリアと誤診されることがあり、専門家は、診断が考慮されない限り重症例でも容易に見逃されると指摘している。

2011年1月、バベシア症は全国的な届出疾患となり、州保健省はバベシア症例に関してCDCと情報を共有することを奨励している。バベシア症に関する正確な情報を得ることは、血液供給をより安全にするために有益である。

報告企業の意見	今後の対応
2011年1月、バベシア症が全米において届出疾患となったことを述べた米国疾病管理予防センターからの報告である。	日本赤十字社では問診時にバベシア症の既往を確認し、該当する場合は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

使用上の注意記載状況・その他参考事項等

解凍赤血球濃厚液「日赤」
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血液を介するウイルス、細菌、原虫等の感染
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JRC2011T-034

Press Release

For Immediate Release: September 6, 2011
Contact: CDC Media Relations
(404) 639-3286

U.S. blood supply vulnerable to parasitic infection spread by ticks

To reduce risk, Babesia test is needed to screen blood donors

Babesia, a tickborne parasite of red blood cells, is being transmitted through blood transfusions, according to results of a collaborative study, led by the Centers for Disease Control and Prevention, of data from the past three decades. Transfusion–associated cases of babesiosis have been increasingly recognized since 1979, the year the first known case occurred.

The article about the study and an accompanying editorial appear today online in the Annals of Internal ɜdicine.

In the report, CDC and collaborators describe 159 transfusion–related babesiosis cases that occurred during 1979–2009, most (77 percent) from 2000 to 2009. No Babesia test approved by the Food and Drug Administration is available for screening prospective blood donors, who can feel fine despite being infected.

Babesiosis is a potentially fatal but treatable complication of transfusion. Severe consequences, such as multi–organ failure and death, are most often seen in persons without a spleen, the elderly, and those with a weak immune system. The study authors say prevention strategies, including development of a screening test, are needed. Some manufacturers are working with investigators at blood establishments to develop FDA–approved tests for Babesia for donor–screening purposes.

"We want clinicians to become more aware of babesiosis, including the small possibility of transmission via blood transfusion," says Barbara Herwaldt, M.D., M.P.H., CDC medical epidemiologist, and lead author of the article. "If a patient develops unexplained fever or hemolytic anemia after a transfusion, babesiosis should be considered as a possible cause, regardless of the season or U.S. region."

Because babesiosis is spread most commonly by ticks, the risk of this disease is another reason for people prevent tick bites. People who unknowingly become infected through the bite of a tiny tick (about the size of a poppy seed) can transmit the parasite via blood transfusion. Therefore, prevention of tickborne infection can help safeguard the blood supply.

Most U.S. tickborne Babesia cases have occurred in seven states in the Northeast and the upper Midwest (in parts of Connecticut, Massachusetts, Minnesota, New Jersey, New York, Rhode Island, and Wisconsin), particularly during the warm months of the year. However, transfusion–associatedBabesia cases have been identified in 19 states and have occurred year–round.

Dr. Herwaldt points out that even severe Babesia cases, not just cases that are asymptomatic or mild, are easily missed unless the diagnosis is considered. Even then, babesiosis often is mistakenly diagnosed as malaria, which also infects red blood cells.

In January 2011, babesiosis became a nationally notifiable disease, which means state health departments are encouraged to share information about cases of babesiosis with CDC. More accurate information about tickborne and transfusion–transmitted cases of babesiosis will help CDC and its partners, including the Food and Drug Administration, in their continued efforts to make the blood supply even safer.

Annals of Internal Medicine
Article: http://www.annals.org/content/early/2011/09/02/0003-4819-155-8-201110180-00362
Editorial: http://www.annals.org/content/early/2011/09/02/0003-4819-155-8-201110180-00363

links below for two government–sponsored events that focused on improving blood safety from babesiosis risk.

Information on babesiosis: http://www.cdc.gov/parasites/babesiosis/index.html

Information on the *Babesia* parasite: http://www.dpd.cdc.gov/dpdx/HTML/Babesiosis.htm

Information on FDA public workshop

Information on the Blood Products Advisory Committee meeting

Information on ticks: http://www.cdc.gov/ticks/

For information on CDC's roles in monitoring blood safety: http://www.cdc.gov/bloodsafety

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Historical Document: September 6, 2011

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Closed Holidays - cdcinfo@cdc.gov

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2011. 7. 14	該当なし	

一般的名称	新鮮凍結人血漿	研究報告の公表状況	百瀬 俊也, 三浦左千夫, 佐藤陽子, 大塚裕司, 平力造, 日髙敏, 北折健次郎, 濱口元洋, 高松純樹, 日野学. 第59回 日本輸血・細胞治療学会総会; 2011.4.14-16; 東京都	公表国
販売名(企業名)	新鮮凍結血漿「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」(日本赤十字社) 新鮮凍結血漿-LR「日赤」成分採血(日本赤十字社)			日本

研究報告の概要

○在日ブラジル人献血者における*Trypanosoma cruzi*抗体検査の研究的試行とシャーガス病に関するアンケート結果について

目的: サシガメを媒介虫とするシャーガス病は中南米諸国で流行し、感染者は*Trypanosoma cruzi*(以下*T.cruzi*)を長期間体内に保有する無症候性のキャリアとなることが知られている。日本でも中南米からの定住者が約40万人いると言われているが、献血者の中にキャリアがどの程度存在するのかは不明である。今回、日本に定住しているブラジルサッカーチームのブラジル人サポーター(多くは日系人)の献血イベントに際し、同意を得た上で*T.cruzi*抗体検査と出身地域等のアンケート調査を実施したので報告する。

対象と方法: 在日ブラジル人グループから愛知県赤十字血液センターへ献血協力の申し込みが事前にあったので、献血イベントに参加した献血希望者に対し、あらかじめ*T.cruzi*抗体検査の研究的試行の説明を行い、同意した者から検体を採血しアンケート調査に協力していただいた。*T.cruzi*抗体検査は迅速法(イムノクロマト法)及びELISA法を同センターにて実施し、追加検査を慶応大学医学部にて行った。その他の献血にかかる問診、検査は通常の献血と同様に実施した。

結果: 献血希望者は20名であり、全員が*T.cruzi*抗体検査の研究的試行に同意した。ELISA法は20名全員陰性であったが、迅速法は19名陰性、1名判定保留であった。追加検査の結果、判定保留の1名は偽陽性であると判断した。20名の献血希望者は、男性13名(うち1名VVRにより不採血)、女性7名(うち4名Hb濃度不足により不採血)であり、年齢は20代9名、30代11名であった。出身地はサンパウロ州17名、パラナ州2名、ミナスジェイラス州1名であった。多くはシャーガス病やサシガメを知っていたが、全員家族にシャーガス病の者はおらず、また過去に*T.cruzi*抗体検査をした者は1名のみであった。

結語: 在日ブラジル人献血希望者20名に対し同意を得てT.cruzi抗体検査を実施した結果、全員陰性であった。20-30代の若い世代であり、家族にもシャーガス病の者はいなかったが、中南米に居住歴を有する献血者へのさらなる検討が必要と考える。

使用上の注意記載状況・その他参考事項等

新鮮凍結血漿「日赤」
新鮮凍結血漿-LR「日赤」
新鮮凍結血漿-LR「日赤」成分採血

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
同意を得た在日ブラジル人献血者20名に対し*T.cruzi*抗体検査を実施したところ、全員が陰性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。

23

O-119 輸血後劇症肝炎にみた感染症検査の問題点と今後の課題

浜松医科大学医学部附属病院輸血細胞治療部[1]，日本赤十字社血液事業本部安全管理課[2]，
日本赤十字社中央研究所感染症解析部[3]，浜松医科大学医学部脳神経外科[4]
山田千亜希[1]，藤原晴美[1]，百瀬俊也[2]，内田茂治[3]，難波宏樹[4]，平松久弥[4]，小川奈緒子[1]，
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【緒言】本邦の輸血医療の進歩は輸血後感染症を激減させたが，改善のために不断の努力が必要な課題である．今回，輸血後劇症肝炎を発症した症例が発生したが，輸血の安全性の更なる向上を図るため，家族の同意を得た上で当大学倫理委員会の指針に従い症例を詳細に検証し，感染症検査の重要性と今後の課題を検討した．【症例】2008年1月，患者は手術に伴いRCC2袋，計3単位を輸血した．輸血67日前に測定したHBs抗原，HCV抗体，HIV抗体は全て陰性であり，輸血直前に遡及調査用検体を採取していた．献血者2名は献血時のHBs抗原，HBc抗体，20プールNAT検査が全て陰性であった．輸血32日後，患者は他施設でHBs抗原を測定し陰性であった．輸血95日後より全身状態の急速な悪化を認め劇症肝炎と診断された．この時の検査でHBs抗原，HBe抗原，HBc抗体，HBV DNAが陽性となった．血漿交換を含む治療が施行されたが，入院後3日で不幸な転帰をとった．患者の輸血前保存検体ではHBs抗体，HBc抗体，個別NAT検査が全て陰性であったが，1名の献血者由来検体の個別NATが陽性となった．献血者及び患者間で両者のウイルスgenotypeは一致した．患者は生物由来製品感染等被害救済制度による救済給付の対象となった．【考察】現行の20プールNATの検出感度は64IU/mlとされ，それ以下のウイルス濃度での肝炎発症が認められたことになる．感度やコスト等にも限界があるが，輸血用血液に対する検査技術の更なる向上が求められる．また現在推奨されている輸血後感染症検査の実施時期（3ヶ月）に肝炎を発症した．感染の早期発見によって抗ウイルス薬による早期治療が可能な場合もあり，症状の軽減化が期待されるため，検査時期を考える上でも貴重な症例である．輸血後感染症検査の重要性を医療従事者に加え患者自身にも理解してもらい，検査の遂行率を上げていく必要がある．

O-120 在日ブラジル人献血者における *Trypanosoma cruzi* 抗体検査の研究的試行とシャーガス病に関するアンケート結果について

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【目的】サシガメを媒介虫とするシャーガス病は中南米諸国で流行し，感染者は *Trypanosoma cruzi*（以下，*T.cruzi*）を長期間体内に保有する無症候性のキャリアとなることが知られている．日本でも中南米からの定住者が約40万人いると言われているが，献血者の中にキャリアがどの程度存在するのかは不明である．今回，日本に定住しているブラジルサッカーチームのブラジル人サポーター（多くは日系人）の献血イベントに際し，同意を得た上で *T.cruzi* 抗体検査と出身地域等のアンケート調査を実施したので報告する．
【対象と方法】在日ブラジル人グループから愛知県赤十字血液センターへ献血協力の申込が事前にあったので，献血イベントに参加した献血希望者に対し，予め *T.cruzi* 抗体検査の研究的試行の説明を行い，同意した者から検体を採血しアンケート調査に協力していただいた．*T.cruzi* 抗体検査は迅速法（イムノクロマト法）及びELISA法を同センターにて実施し，追加検査を慶應大学医学部にて行った．その他の献血に係る問診，検査は通常の献血と同様に実施した．
【結果】献血希望者は20名であり，全員が *T.cruzi* 抗体検査の研究的試行に同意した．ELISA法は20名全員陰性であったが，迅速法は19名陰性，1名判定保留であった．追加検査の結果，判定保留の1名は偽陽性であると判断した．20名の献血希望者は，男性13名（うち1名VVRにより不採血），女性7名（うち4名Hb濃度不足により不採血）であり，年齢は20代9名，30代11名であった．出身地はサンパウロ州17名，パラナ州2名，ミナスジェイラス州1名であった．多くはシャーガス病やサシガメを知っていたが，全員家族にシャーガス病の者はおらず，また過去に *T.cruzi* 抗体検査をした者は1名のみであった．
【結語】在日ブラジル人献血希望者20名に対し同意を得て *T.cruzi* 抗体検査を実施した結果，全員陰性であった．20-30代の若い世代であり，家族にもシャーガス病の者はいなかったが，中南米に居住歴を有する献血者へのさらなる検討が必要と考える．

医薬品
医薬部外品　　研究報告　調査報告書
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一般的名称	①②乾燥抗 HBs 人免疫グロブリン ③ポリエチレングリコール処理抗 HBs 人免疫グロブリン	研究報告の公表状況	http://www.hhs.gov//2011/07/27	公表国 アメリカ
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-180-

研究報告の概要

FDA は、サウジアラビアで BSE 原因物質に感染したと考えられるヒトで vCJD が 3 例発生したことに基づき、血液及び血液成分（原料血漿を含む）のドナーならびにヒト細胞、組織、及び細胞と組織をベースとした製剤（HCT/P）のドナーについての現行の vCJD 関連の安全性勧告事項を改変すべきか否か、そしてサウジアラビアでの滞在期間について、特定の血液ドナーを不適格とすることの勧告、または HCT/P のドナーで特定の不適格なドナーを見出すことに関する勧告について、伝達性海綿状脳症諮問委員会（TSEAC）からの助言を求めている。

2011 年 3 月に Health Canada は最近移住してきた 1 人が vCJD のほぼ確実な例であるとの報告を行った。その患者は若い男性で 1986 年生まれ、少年期は殆どサウジアラビアで過ごしており（12 年間）、その後隣接するドバイ（UAE）で 4 年間を過ごした。この患者の vCJD の最初の症状は、ドバイからカナダへ移住する前の 2011 年初期に現れていた。Health Canada の専門家は、その男性はこれまで手術を受けたことも輸血を受けたこともないので、おそらくはアラビア半島に居住していた間に、さらに言えばドバイよりもこの患者が少年期の殆どを過ごしていたサウジアラビアで、BSE 原因物質に食物を介して暴露されて感染したのであろう、と結論づけている。この患者はサウジアラビアで食物を介して BSE 原因物質に暴露されたと考えられる第 3 例目である。以前に報告された vCJD の 1 例は、UCSF 病院（University of California, San Francisco）で診断され、概略が CDC に報告された例で、ヴァージニア州に住んでいた患者で、サウジアラビア人ではないが、サウジアラビアで生まれ育っていた。更にそれ以前の 2003 年の vCJD 症例は、詳細な報告はないが、33 歳のサウジアラビア人で、おそらくはサウジアラビア国内で感染したものであろうと結論づけた。
サウジアラビアから世界動物保健機関（World Organisation of Animal Health）への BSE の症例報告はこれまで全くない。しかし、サウジアラビアは問題となっていた期間（1980 年～1996 年）に英国からウシ及び牛肉製品を輸入していたことが確認されており、またサウジアラビアは肉骨粉（MBM）が BSE 原因物質によって汚染されていた可能性があるとされている 1988～1993 年の間に、英国起原の MBM の荷受け先であったことが確認されている。我々は、サウジアラビアでの BSE 原因物質へのヒトへの暴露危険性は、懸念されている年度である 1980 年から 1996 年までの間にこの地域に輸出された英国起原の牛肉及び生牛が、主要なものであると推測した。

サウジアラビアに居住していたことによるものと考えられる vCJD の 3 例の報告は、米国の血液安全性についての勧告事項や HCP/P の安全性に影響を与えるが、それはドナーとなる可能性のある次の 4 群の人々の適格性に影響を及ぼす：

1980-1996 年の間に (1) サウジアラビアに駐留していた米軍関係者
(2) サウジアラビアの米軍活動をサポートしていた契約請負業者の米国人労働者
(3) サウジアラビアで非軍事の請負業者に雇われていた米国人労働者
(4) サウジアラビアに住んでいたが米国に移住した移住者

現在のところサウジアラビアは、FDA がドナーの不適格性を判断することを勧告している国々のリストには含まれていない。FDA は現行の適格性/不適格性の勧告事項を改定してその中に、1980 年から 1996 年末までの間にサウジアラビアに軍関係者として累積で 6 ヶ月以上滞在した、血液及び血液成分（原料血漿及び HCT/P を含む）のドナーを含めることを考慮中である。

使用上の注意記載状況・
その他参考事項等

代表としてヘブスブリン IH 静注 1000 単位の記載を示す。
2. 重要な基本的注意
(1)略
1)略
2)現在までに本剤の投与により変異型クロイツフェルト・ヤコブ病（vCJD）等が伝播したとの報告はない。しかしながら、製造工程において異常プリオンを低減し得るとの報告があるものの、理論的なvCJD等の伝播のリスクを完全には排除できないので、投与の際には患者への説明を十分行い、治療上の必要性を十分検討の上投与すること。

24

医薬品
医薬部外品　　研究報告　調査報告書
化粧品

	議題： 1. 以下の者について、原血漿を含む血液と血液製剤のドナーの延期を推奨、及び HCT/P の不適格なドナーとする a) 1980 年初め～1996 年末までの間、米軍としてサウジアラビアに累積 6 ヵ月以上滞在した者 b) 1980 年初め～1996 年末までの間、サウジアラビアに累積 5 年以上滞在した者 2. 血液、血液製剤、血漿由来品、HCT/P の供給の影響の可能性、製品の安全性についてのこれら推奨事項の寄与について 3. FDA の案、または更なる安全性基準について TSEAC は、サウジアラビアを訪れた一部ドナー（1980 年初めから 1996 年末まで米国軍人としてサウジアラビアに累計 6 ヵ月以上滞在していた人、或いは同期間に累計 5 年間以上滞在していた人）は献血延期すべきであることについて合意した。	

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

FDA Transmissible Spongiform Encephalopathies Advisory Committee (TSEAC)
23rd Meeting, August 1, 2011
Gaithersburg, MD

Issue Summary

Donor Deferral/Ineligibility for Time Spent in Saudi Arabia to Reduce Risk of vCJD Transmitted by Blood and Blood Products and by Human Cells, Tissues and *Cellular and Tissue-Based Products (HCT/Ps)*

Issue

FDA seeks advice from TSEAC on whether, based on three cases of vCJD in individuals likely to have been infected with the BSE agent in Saudi Arabia, to modify current vCJD-related safety recommendations for donors of blood and blood components, including Source Plasma, and for donors of HCT/Ps, to recommend deferring of certain blood donors or finding ineligible certain donors of HCT/Ps for time spent in Saudi Arabia.

Background

vCJD in recent Saudi immigrant to Canada. In March 2011 Health Canada described a probable case of vCJD in a recent immigrant. The diagnosis of vCJD was supported by results of a tonsil biopsy in Canada showing accumulation of abnormal prion protein [26]. The patient is a young man, born in 1986, who spent most of his early life in Saudi Arabia (12 yr) and, later, in neighboring Dubai, United Arab Emirates (4 yr) [4, 11]. He first showed symptoms of vCJD early in 2011 before emigrating from Dubai to Canada. The probable time of food-borne infection with the BSE agent for individuals with the patient's prion-protein-encoding (*PRNP*) genotype (129MM) falls within the years he lived in the Arabian Peninsula (estimated median incubation period of food-borne vCJD for persons with the *PRNP*-129-MM genotype, estimated to be 12 to 13 yr [1, 18]). Authorities at Health Canada [19] have concluded that the man, who has no history of surgery or blood transfusion, was probably infected by dietary exposure to the BSE agent while in the Arabian Peninsula, more likely in Saudi Arabia, where he spent most of his early years, than in Dubai. It is much less likely, though not impossible, that he might have been exposed to the BSE agent during a visit of two weeks to the UK in 1995 (near the end of the UK BSE dietary risk period). He paid a second brief visit to the UK in 2002, six years after the UK dietary risk period is thought to have ended [25]. This is the third case of vCJD plausibly attributed to a dietary exposure to BSE agent in Saudi Arabia.

vCJD in other persons born in Saudi Arabia. One previous case of vCJD, diagnosed by brain biopsy at the UCSF Hospital, University of California, San Francisco, and briefly described by the CDC, occurred in a person living in Virginia who was a non-Saudi Arabian national born and raised in Saudi Arabia [5]. That person's family recalled no history of travel to UK except for connecting flights. An earlier case of vCJD in 2003, never described in detail, affected a 33-year-old Saudi citizen who underwent brain biopsy at a hospital in Saudi Arabia; vCJD was diagnosed from a sample of the biopsy sent to the Mayo Clinic, Rochester, Minnesota, and confirmed by the National Prion Disease Surveillance Center, Case Western Reserve University, Cleveland, Ohio [1]; CDC noted that the patient "may have visited the UK, if at all, only for several days"

(although he had visited France) and concluded that he was most likely to have been infected in Saudi Arabia [5].

BSE in Saudi Arabia. Saudi Arabia has not reported any case of BSE to the World Organisation for Animal Health (OIE) [36]. BSE has very rarely been recognized in other countries of the general region: two cases of BSE were reported in cattle imported into Oman in 1989 [37] and one case in a native bovine in Israel was reported in 2002 [38]. However, Saudi Arabia was identified as having imported live cattle and beef products from the UK during the period of concern (1980-1996) [24], and Saudi Arabia was identified as a consignee of meat-and-bone meal (MBM) of UK origin, during the years 1988-1993 when MBM might have been contaminated with the BSE agent [23].

We have assumed, for the purposes of this analysis, that the major risk of human exposure to the BSE agent in Saudi Arabia was from beef and live cattle of UK origin exported to the region during the years of concern: 1980 through 1996. According to Sanchez-Juan and colleagues [24], the UK exported to Saudi Arabia almost 1,000 live bovines (1980-1990) and about 32,000 tons of carcass meat (1980-1996). Earlier estimates of exports reported by a representative of the World Health Organization (WHO) to TSEAC were roughly similar [23]. However, we cannot verify the accuracy of those figures. We have also assumed that exports of live cattle, beef, MBM and other bovine-derived products exports from the UK to Saudi Arabia ceased when the European Commission prohibited such exports both to Member States of the European Community and to "third countries" in March 1996 [16]. Furthermore, the UK implemented an enhanced prohibition of mammalian proteins in ruminant feed in 1996 and other controls to enhance the safety of food for humans and animal feeds by the end of 1996 [25]; therefore, we conclude that the risk of exposure to the BSE agent in any products and live cattle exported from the UK to Saudi Arabia after that time was small.

We acknowledge that other BSE countries (i.e., countries of Europe) might also have exported beef to Saudi Arabia and neighboring countries both during the years 1980 through 1996 and afterwards, however (1) the much lower rates of both diagnosed BSE and vCJD cases in other countries relative to the UK suggest that the risk associated with beef from those countries must be considerably less than for UK beef, and (2) we have not been able to estimate imports of beef from non-UK countries into Saudi Arabia. We are also unable to estimate cross-border sales of cattle or beef products in the region or the possibility that BSE might have been introduced into native ruminants in Saudi Arabia by the use of MBM—either imported or domestically produced—in animal feed supplements. Saudi Arabia is estimated to have had about half a million cattle in 1998 and far larger numbers of camels, goats, and sheep [2]. While acknowledging the theoretical possibility of BSE infections in local ruminants, we concluded that the risk of such infections is probably much less than that of beef products from the UK and too uncertain to consider unless and until reliable information becomes available.

Estimating the possible risk of dietary exposure to the BSE agent in US donors of blood and tissues during residence in Saudi Arabia. Since 1999, FDA's recommendations regarding deferral of blood and ineligibility of donors of HCT/Ps potentially exposed to the BSE agent in various countries—geographic deferrals—have been based on rough comparisons of the estimated risk of oral exposure to the BSE agent

in various groups of people compared to the risk of the UK population from the beginning of 1980 until the end of 1996, when UK food/feed protections were fully implemented. FDA, in 2001, announced a model that estimated the risk in most countries of Western Europe assigned as a relative-risk compared to the UK. The risk of dietary exposure to the BSE agent was assumed to be stochastic and directly (linearly) related to the time spent in a country where the BSE agent contaminated beef products [6]. In principle, the exposure of concern was consumption of beef products, but dietary histories were unavailable and are probably unreliable, so donor days in country were taken as a surrogate. Based on a number of other assumptions, the following relative risks were assigned: UK=1.0, France=0.05 (i.e., 5% of beef in France assumed to have been imported from the UK [3] and other countries of Western Europe=0.015 (extrapolating to the rest of Western Europe the results of intensive surveillance of BSE in Switzerland) [6]. For purposes of deferral policy, and in consideration of the absence of more detailed information, vCJD risk in Western Europe was taken as comparable to that in France as a worst case. A risk relative to UK of 0.35 was assigned to US military bases that obtained beef from the UK in various years using estimates of UK beef sourcing provided to the FDA by the US Department of Defense (DoD) [9]. These estimated relative risk factors are highly uncertain because of uncertain simplifying assumptions that underlie them. In fact, the model appears to have predicted fewer cases of vCJD than have been recognized in France (25 to date or more than 10% of the UK per capita rate) and overestimated cases in US military personnel and dependents (no cases to date among as many as 4.8 million active duty personnel and an unknown number of dependents and employees [32]). At the time, FDA also attempted to predict the possible loss of otherwise suitable blood donors that might result from various vCJD-related geographic donor deferral policies, based on a travel survey of donors in 12 blood centers [6]. Insofar as limited information has been available to us, we attempted a similar assessment of vCJD-related risk in Saudi Arabia, an assessment of reduction in that risk by donor deferral policies, and an estimation of the possible loss of otherwise suitable blood donors that might result.

1) **Estimates of relative prevalence of vCJD in various countries compared with Saudi Arabia.** We attempted to estimate vCJD risk in donors resident in Saudi Arabia by comparing the crude rate of vCJD attributed to residence in Saudi Arabia with rates for seven European countries currently on the FDA deferral list that have had cases attributed to infection within the country, not including three cases attributed to infection during residence in the UK [27]. Information to date, summarized in **Table 1,** suggests that the crude recognized prevalence of vCJD attributed to exposure to the BSE agent in Saudi Arabia to date (three cases in a total population estimated by the US Census Bureau earlier this year to be 26,132,000 [29]) resembles that in a number of European countries (somewhat lower than estimated prevalences in Ireland and France, both of which have lower rates than UK) for which FDA currently recommends geographic deferrals of blood donors [9] and screening of HCT/P donors [8]. It is important to note that the crude prevalence estimates provided in Table 1 have not been adjusted either for ages of the populations (younger persons being more often affected by vCJD than older persons) in the different countries or for probable differences in vCJD case recognition and reporting.

2) **Potential consumption of UK beef products by persons resident in Saudi Arabia 1980-1996.** In trying to estimate exposure to UK beef products, we addressed two groups of residents.

(a) Estimated consumption of UK beef by the general population of Saudi Arabia, including Saudi nationals and foreign residents. We considered two factors affecting the risk of dietary exposure to the BSE agent: **(i)** estimated UK exports of beef to Saudi Arabia during the years 1980 through 1996, and **(ii)** estimated total beef consumption in Saudi Arabia. The latter adjustment was based on published data reporting that residents of Saudi Arabia, on average, consume considerably less beef than do residents of the UK and other Western European countries. Published sources suggested that about 10% of beef imported into Saudi Arabia during the years of concern might have originated in the UK [24] [31] and that average annual per capita beef consumption in Saudi Arabia was about a quarter of that in the UK (lamb and poultry being more popular) [2, 17, 30]. Taken together, these figures, although not validated and admittedly uncertain, suggested that a reasonable average relative risk estimate for dietary exposure to BSE agent in UK beef by persons resident in Saudi Arabia during the years 1980-1996 might be 0.025 (i.e., 0.10 x 0.25) that of persons resident in the UK during the same period and not unlike the risk previously estimated for most countries of Western Europe.

(b) US military personnel on bases in Saudi Arabia. We also considered information provided to FDA by the Armed Services Blood Program Office, DoD, about sources of beef supplied by the US Government to the US military personnel stationed in countries of the Arabian Peninsula during the years of concern (which include the years of the First Persian Gulf War). Information about military beef was taken from a recent DoD review of procurement records. Beef in field rations/"meals ready to eat" [MREs] during those years was all of US origin. However, an uncertain but possibly significant amount of the beef sold to and consumed by US military personnel living on US bases in Europe and Saudi Arabia after 1980 originated in the UK, though such procurement decreased after 1989. We cannot assume with confidence that the origin of beef consumed on US bases in Saudi Arabia differed significantly from that on European bases south of the Alps. Acknowledging the uncertainties, we therefore assumed that the risk of dietary exposure to the BSE agent for US military personnel living on bases in Saudi Arabia from 1980 through the end of 1996 was similar to that FDA previously assigned to US military living on European bases south of the Alps, taken to be about 35% of that for UK residents during the same period. Unlike US military stationed on European bases, no military dependents lived in Saudi Arabia. For the most part, US military contractors were not supplied with food by DoD, purchased their food locally—"on the economy"—and so are assumed to have shared the general dietary risk of exposure to the BSE agent with other residents of Saudi Arabia.

Canadian deferral of blood donors resident in Saudi Arabia. Since November 2007, Héma-Québec [21], a blood establishment operating in the Province of Quebec, has requested deferral of blood donors resident in Saudi Arabia for any period of six months

or more from 1980 through 1996 [20]. Since March 2011, Canadian Blood Services (CBS) has required the same deferral [19]. Canadian blood donor deferral policies for residents of Saudi Arabia do not include donors with history of blood transfusion in that country. **Table 2a** compares current Canadian and US blood donor deferral policies for vCJD risk. The policies, while similar, are not identical. We are not aware that any other country has recommended blood donor deferral for residents of Saudi Arabia.

Canadian assessments for determining suitability of donors of cells and tissues. Health Canada requires that travel information be collected for cell and tissue donors and some other questioning of donors or their proxies about vCJD risk factors. There are, however, no exclusion criteria based on risk factors associated with residence in or travel to specific geographic areas [19]. US donor screening recommendations regarding vCJD for donors of HCT/Ps are summarized in **Table 2b.**

FDA's proposed response to reports of three vCJD cases in individuals likely to have been infected with the BSE agent in Saudi Arabia. The reports of three cases of vCJD attributed to residence in Saudi Arabia has implications for US blood safety recommendations and for the safety of HCT/Ps, affecting the suitability of four groups of potential donors: US military personnel serving in Saudi Arabia, US guest workers who were military contractors supporting US forces in Saudi Arabia, other US guest workers employed as non-military contractors in Saudi Arabia and immigrants to the US who lived in Saudi Arabia, during the years 1980-1996. Saudi Arabia is not currently included on the list of countries for which FDA has recommended deferral/ineligibility of donors [9].

FDA is considering modifications to current suitability/eligibility recommendations to include donors of blood and blood components, including Source Plasma and HCT/Ps who spent any cumulative period of six months or longer as military personnel serving in Saudi Arabia from 1980 through the end of 1996. This recommendation is similar to the current recommendation to defer donors resident on US military bases in Europe during years when they were supplied with UK beef (comprising an estimated 35% of the beef supply through 1996 south of the Alps [9]). FDA is also considering modifications to current suitability/eligibility recommendations to include any other donors of blood and blood components, including Source Plasma and HCT/Ps who spent any cumulative period of five years or longer living in Saudi Arabia from 1980 through the end of 1996. This modification is similar to the current recommendation to defer donors resident in France, except that, because of continuing reports of BSE affecting native cattle in several European countries and a lack of reliable information regarding implementation of food safety measures and cross-border trade in beef products in Europe [7], FDA continues to consider the period of potential dietary exposure to the BSE agent for France and most other European countries (except UK) to extend to the present. We have assumed that the BSE risk for Saudi Arabia was associated with importation of live cattle and beef from the UK and that the risk became negligible at the end of 1996.

We acknowledge that Saudi Arabia might have imported live bovines and beef from other BSE countries after 1996 [2], but we have not included that assumption in developing the proposed recommendations. Saudi Arabian authorities have assured FDA that, since at least 1996, the Kingdom has prohibited the importation of live bovines and

beef products from countries reporting BSE to the OIE, as suggested by public sources [14]. As noted above, Saudi Arabia has reported no case of BSE to the OIE, and we assume that native Saudi cattle have probably not been infected. The likelihood that BSE infection was established in the substantial number of small ruminants (sheep and goats—far outnumbering cattle in Saudi Arabia [2, 17]) seems remote. We do not have information regarding rendering and animal feeding practices in Saudi Arabia (specifically on production of MBM and use of MBM in feeds) that would allow more reliable assumptions.

Potential impact on US blood supply and on HCT/P supply resulting from proposed deferral of certain blood donors or ineligibility of certain cell and tissue donors resident in Saudi Arabia during the years 1980-1996

We considered four potential at-risk groups that would be affected under the proposed recommendation for US donors with a history of residence in Saudi Arabia during the years 1980-1996. The groups include: (1) US military personnel; (2) US guest workers who were contractors to the US military; (3) US guest workers who were contractors but not for the US military; and (4) immigrants from Saudi Arabia to the US (both Saudi and non-Saudi nationals, regardless of current citizenship). **Table 3** below summarizes the predicted number of US donors and blood donations lost as a result of the proposed changes to recommendations for determining suitability of blood and plasma donors based on residence in certain countries. Because the more limited available information on donors and donations of HCT/Ps, FDA has not been able to analyze the possible impact of the proposed recommendation on the US supply of HCT/Ps.

US military personnel. Based on information provided to FDA by DoD, approximately 600,000 US troops were deployed to Saudi Arabia for a period ≥ 6 months in the years 1980-1996; that number represents about 90% of total deployments to Saudi Arabia during that period. Those persons would all be deferred from blood donations or ineligible to donate under the proposed geographic risk factor recommendations. However, DoD estimates that approximately 30% of this population are already deferred from donating due to the vCJD European deferral and other reasons. In addition, a large number of this population retired or left the military and may be donating to civilian blood collection facilities.

US military contractor guest workers. Information from DoD indicated that approximately 200,000 personnel including DoD civilians and contractors were employed in Saudi Arabia during the years 1980-1996. We assumed that all had cumulative stays of ≥ 6 months but less than 5 years. Under the proposed recommendation, they would not be deferred from donating blood and would remain eligible to donate HCT/Ps.

US non-military contractor guest workers. We assumed all US non-military guest workers who lived in Saudi Arabia during the years 1980-1996 had cumulative stays ≥ 6 months with an average length of stay of four years [10, 12, 13, 15, 22]. We further assumed that 30% of US guest workers lived in Saudi Arabia for more than 5 yr [10, 12, 13, 15, 22] and thus would be deferred from blood donation and ineligible to donate HCT/Ps under the proposed geographic BSE risk factor recommendations. The *Average Annual Number* of US guest workers in Saudi Arabia was estimated using data from the

US State Department on the number of registered US citizens in Saudi Arabia in 1999 [35]. We used these data for the year 1999, extrapolated and summed each year to derive the total number of US guest workers in Saudi Arabia during 1980 and 1996.

Immigrants. Our estimates assume that all immigrants from Saudi Arabia since 1985 had stayed for ≥ 5 yr in Saudi Arabia during the years 1980-1996, and they would be deferred from blood donations or ineligible to donate under the proposed geographic BSE risk factor recommendations. The *Average Annual Number* of persons emigrating from Saudi Arabia to the US from 1985 to the present was derived from Immigration Statistics 1989-2010 released by the US Department of Homeland Security [34]. Our estimates do not capture non-Saudi nationals immigrating to the US from Saudi Arabia as the last residence of record and thus may somewhat underestimate the number of donors and donations in this category that would actually be lost.

Donor loss calculation. We calculated blood donor loss based on the assumption that individuals who resided in Saudi Arabia during the years 1980-1996 have a 5% rate of donation [28, 33], which is the donation rate for the general US population. Our calculation for the potential loss of blood units assumes that each donor donates approximately 1.7 units of blood each year [33]. The estimated potential impact on US blood supply resulting from the proposed donor deferral recommendation is summarized in Table 3

Questions for TSEAC

Question 1. Do available data support the consideration by FDA to recommend deferring donors of blood and blood components, including Source Plasma, and to determine to be ineligible donors of HCT/Ps, who

a) spent six months or more cumulatively in Saudi Arabia as US military personnel from the beginning of 1980 through the end of 1996 or

b) otherwise spent more than five years cumulatively in Saudi Arabia from the beginning of 1980 through the end of 1996?

Question 2. Please discuss the likely contribution of those recommendations to the safety of the products involved and the possible impact on supplies of blood, blood components, plasma derivatives and HCT/Ps.

Question 3. Please comment on additional information that might better inform FDA's consideration of the proposed or any further safety measures.

Table 1.

Reported vCJD cases per estimated total population 2011

Country	vCJD Cases	Estimated* Population 2011	Crude Rate
UK	175	62,698,362	2.8×10^{-6}
Ireland	2	4,670,849	4.3×10^{-7}
France	24	65,102,719	3.7×10^{-7}
Portugal	2	10,760,305	1.9×10^{-7}
Netherlands	3	16,847,007	1.8×10^{-7}
Spain	5	46,754,657	1.1×10^{-7}
Saudi Arabia	3	26,131,703	1.1×10^{-7}
Italy	2	61,016,804	3.3×10^{-8}
Japan	1	126,475,664	7.9×10^{-9}

vCJD cases are attributed to exposure in a country according to the conclusion of the CJD Surveillance Unit, Edinburgh [27]. Cases resident for ≥ 6 mo in UK are attributed to UK. Rates are not adjusted for differences in population age profiles or for efficiency of case recognition and reporting in various countries.

*Population estimates for various countries were taken from the Web site of the US Census Bureau for mid-year 2011 [28].

Table 2a

Comparison of Geographic vCJD-related Blood Donor Deferral Policies Recommended by FDA and Required by Canadian Blood Services

	USA FDA	Canadian Blood Services	Héma-Québec
UK	≥ 3 mo 1980-1996[a]	≥ 3 mo 1980-1996[b]	≥ 1 mo 1980-1996
France	≥ 5 yr 1980-present	≥ 3 mo 1980-96	≥ 3 mo 1980-96
Other Western Europe (WE)	≥ 5 yr 1980-present ≥ **28** countries[a]	≥ 5 yr 1980-present **12** countries[b]	≥ 6 mo 1980-present **12** countries[c]
Transfusion history	UK, France 1980-present	UK, France, WE 1980-present	UK, France, WE 1980-present
Saudi Arabia	no deferral	≥ 6 mo 1980-1996	≥ 6 mo 1980-1996
Other countries	no deferral	no deferral	no deferral

a US definition of United Kingdom = England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, and the Falkland Islands

US definition of WE (excluding UK, France) = Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Demark, Finland, Germany, Greece, Hungary, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, and the Federal Republic of Yugoslavia ([sic] now Kosovo, Montenegro, and Serbia)

FDA also recommends deferral of US military personnel who spent ≥ 6 mo on certain military bases in Europe 1980-1996 [9]

b Canadian definition of United Kingdom = England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands (excludes Gibraltar and the Falkland Islands). Canadian definition of WE (excluding UK, France) = Austria, Belgium, Demark, Germany, Republic of Ireland, Italy, Liechtenstein, Luxembourg, Netherlands, Portugal, Spain, Switzerland

c http://www.hema-quebec.qc.ca/donner/don-de-sang/qui-peut-donner-du-sang/creutzfeldt-jakob.en.html

Table 2b.

Geographic vCJD-related Cell and Tissue Donor Eligibility Policies Recommended by FDA

Ineligible Donors with history of	USA FDA
Residence in UK	≥ 3 mo 1980-1996[a]
Residence in Other Western Europe	≥ 5 yr 1980-present ≥ **28** countries[b]
Blood transfusion	UK, France 1980-present
Residence in Saudi Arabia	no current recommendation
Residence in Other Countries	no current recommendation

a US definition of United Kingdom = England, Northern Ireland, Scotland, Wales, the Isle of Man, the Channel Islands, Gibraltar, the Falkland Islands

b US definition of Europe = Albania, Austria, Belgium, Bosnia-Herzegovina, Bulgaria, Croatia, Czech Republic, Demark, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Liechtenstein, Luxembourg, Macedonia, Netherlands, Norway, Poland, Portugal, Romania, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, United Kingdom, Federal Republic of Yugoslavia ([sic] now Kosovo, Montenegro, Serbia)

FDA also recommends deferral of US military personnel who spent ≥ 6 mo on certain military bases in Europe 1980-1996 [8]

Note: Health Canada requires that travel information be collected and some donor other screening for vCJD-related risk factors. There are, however, no exclusion criteria for cell and tissue donors based upon risk factors associated with residence or travel history to specific geographic areas.

Table 3.

Estimated loss of blood donors and blood donations resulting from proposed recommendations to defer certain blood donors with history of residence in Saudi Arabia during 1980-1996

	US Military Personnel	US Guest Workers Military Contractors	US Guest Workers Non-Military Contractors	Immigrants to US	Total
Average annual number	n/a	n/a	36,000[a]	920[b]	n/a
Population to be deferred	420,000[c]	0[d]	45,900[e]	24,800[f]	490,000
Blood donors lost[g]	21,000	0	2,300	1,200	24,500
Blood units lost[h]	35,700	0	3,910	2,040	41,700

Notes:

[a]Average annual number of US guest workers in Saudi Arabia: based on data from US State Department for registered US Citizens living in Saudi Arabia in 1999 (http://overseasdigest.com/amcit_nu2.htm).

[b]Average annual number of immigrants from Saudi Arabia: based on data from US Department of Homeland Security, Yearbooks of Immigration Statistics 2004 and 2010 (http://www.dhs.gov/files/statistics/publications/yearbook.shtm).

[c]Number of military personnel to be deferred calculated by:
600,000 (total number of military personnel who stay for ≥ 6 months, DoD 2011) x 70% (percentage individuals having already been deferred, DoD 2011)

[d]Total number of military contractors who stay for ≥ 5 years, and thus to be deferred (DoD, 2011)

[e]Total number of US guest workers non-military contractors to be deferred calculated by:
(Average Annual Number of US guest workers x 17 years (from January 1, 1980 to December 31, 1996) / Average Length of Stay) x 30% (percent stay for ≥ 5years)

[f]Total number of immigrants to be deferred, calculated by:
Average Annual Number of immigrants x 27 years (from 1985 to current)

[g]Blood donors lost, calculated by:
Population to be deferred x 5% (donation rate)

[h]Number of blood units lost, calculated by:
Number of donors to be deferred x 1.7 (average number of donations per donor per year)

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医薬品
医薬部外品　　研究報告　調査報告書
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研究報告の概要

英国血友病センター医師機構(UKHCDO)が、787 人の先天性出血性疾患患者の血漿製剤による vCJD 感染リスクを評価した。
患者は、供血後に vCJD を発症したドナー由来血漿を含む 1987-1999 年に製造した 25 バッチの何れかの製剤を投与された。これらのバッチの vCJD 感染性を血漿の画分の感染性とバッチの製造データから推測した。各患者の受けた総 vCJD 感染性は、薬剤の総投与量から推測される累積感染性から推算した。
787 人の内、604 人(77%)は汚染バッチの投与を受けてから 13 年間以上追跡調査した。この 604 人の推定 vCJD リスクは、595 人が 1%以上、164 人が 50%以上、及び 51 人が 100%。これらのリスクが英国人の背景的リスクである食事によるリスクに上乗せされる。604 人の患者の内、94 人(16%)は vCJD を発症した患者由来のバッチを、供血後 6 カ月以内に投与された。151 人(25%)は 10 歳以下で製剤の投与を受けていた。
2009 年 1 月 1 日現在、これらの患者は一人も vCJD を発症していない。血漿画分の感染性を過度に見積もったか、輸血用血液成分の受血者よりも潜伏期間が長いことを示唆する。

使用上の注意記載状況・その他参考事項等

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

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

Haemophilia

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ORIGINAL ARTICLE *Transfusion transmitted disease*

The risk of variant Creutzfeldt-Jakob disease among UK patients with bleeding disorders, known to have received potentially contaminated plasma products

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Summary. The risk of variant Creutzfeldt-Jakob disease (vCJD) from potentially infected plasma products remains unquantified. This risk has been assessed for 787 UK patients with an inherited bleeding disorder prospectively followed-up for 10–20 years through the UK Haemophilia Centre Doctors' Organisation (UKHCDO) Surveillance Study. These patients had been treated with any of 25 'implicated' clotting factor batches from 1987 to 1999, which included in their manufacture, plasma from eight donors who subsequently developed clinical vCJD. Variant CJD infectivity of these batches was estimated using plasma fraction infectivity estimates and batch-manufacturing data. Total potential vCJD infectivity received by each patient has been estimated by cumulating estimated infectivity from all doses received during their lifetime. Of 787 patients, 604 (77%) were followed-up for over 13 years following exposure to an implicated batch. For these 604 patients, the estimated vCJD risk is ≥1% for 595, ≥50% for 164 and 100% for 51. This is additional to background UK population risk due to dietary exposure. Of 604 patients, 94 (16%) received implicated batches linked to donors who developed clinical vCJD within 6 months of their donations. One hundred and fifty-one (25%) had received their first dose when under 10 years of age. By 1st January 2009, none of these patients had developed clinical vCJD. The absence of clinical vCJD cases in this cohort to date suggests that either plasma fraction infectivity estimates are overly precautionary, or the incubation period is longer for this cohort than for implicated cellular blood product recipients. Further follow-up of this cohort is needed.

Keywords: haemophilia, inherited bleeding disorders, risk assessment, UK plasma products, variant Creutzfeldt-Jakob disease

Introduction

The bovine spongiform encephalopathy (BSE) epidemic in UK cattle occurred from 1980 to 1996. Evidence has been presented that a distinct clinicopathological variant of Creutzfeldt-Jakob disease (vCJD), first described in 1996 [1], is the human manifestation of BSE [2–4]. Concerns that vCJD may be transmissible by blood and blood products, and actions taken to reduce the risk to UK patients with an inherited bleeding disorder, have recently been reported [5].

The vCJD risks from plasma products linked to donors who later developed vCJD, remain unquantified. Det Norske Veritas's (DNV) risk assessment informed the introduction of further public health measures for recipients of UK-sourced plasma products in 2004 [6]. These recipients included patients with inherited bleeding disorders who had been treated with UK-sourced plasma products between 1980 and 2001. On the advice of the CJD Incidents Panel (CJDIP), and facilitated by the Health Protection Agency (HPA), these patients were informed of their risk by the UK Haemophilia Centres Doctors' Organisation (UKHCDO) *via* their

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Haemophilia Centres and asked to implement public health measures to reduce the possible risk of vCJD spreading to others [5].

The nature of the blood-associated vCJD agent and the impact of processing technologies on the nature and distribution of vCJD infectivity in human blood components and plasma products were unknown. Therefore, the DNV risk assessment was based on data from published animal studies and a number of assumptions [6]. Three options were developed: (i) the fractionation step with the largest clearance of infectivity represents the entire process, (ii) the reduction in infectivity when separating blood into blood components and plasma fractions is the only step that reduces infectivity when producing plasma products, and (iii) the infectivity level correlates with the protein content of plasma products. Option (iii) was rejected as it was considered scientifically invalid. The CJDIP adopted option (ii) rather than (i) on the basis that it was more precautionary and because there were uncertainties around the clearance values in option (i).

This article presents the application of this risk assessment to 787 bleeding disorder patients who have received implicated clotting factor batches linked to donors who later developed clinical vCJD. The identification of the abnormal prion protein associated with asymptomatic vCJD postmortem in a patient in this cohort has prompted this assessment [7]. The implications to inform further public health responses are discussed.

Materials and methods

Implicated plasma product batches

In the UK, a total of 178 plasma product batches have been linked to 25 plasma donations from 11 donors who subsequently developed clinical vCJD [8]. These include 25 implicated clotting factor[1] batches linked to 18 plasma donations from eight donors that have been used to treat 787 UK patients with inherited bleeding disorders. The batches had expired before the 2004 patient notification.

Calculation of infectivity of plasma products

Plasma from many thousands of donations is pooled prior to fractionation. The DNV risk assessment provided estimates of potential infectivity of different plasma fractions. Infectivity was quantified using the ID_{50}, where one ID_{50} is the dose required to produce infection in 50% of recipients.

[1]Factor concentrates are made from pooled plasma and include FVIII, FIX, FVII, FXI, FXIII and prothrombin complex concentrates as well as antithrombin.

In 2004, the HPA used a 'Product Risk Calculator' tool to estimate the infectivity of each implicated batch (Appendix 1 Supporting information). The tool combined the DNV infectivity estimates with fractionators' batch-manufacturing data. For each batch, it calculates the dose estimated to contain 0.02 ID_{50}. This represents a 1% risk of infection in addition to the general background population risk from potential dietary exposure. This is the level of risk the CJDIP considered sufficient to warrant patient notification and public health action [9]. The cumulated lifetime infectivity received by each patient was estimated using the data on each batch and the total quantity received.

Identification and management of patients with bleeding disorders

A policy decision was taken that all bleeding disorder patients treated with UK-sourced clotting factors from 1980 to 2001 (rather than just those who had received implicated clotting factors) should be considered 'at risk' of vCJD for public health purposes [5]. This decision was made because: (i) a single dose of implicated clotting factor was thought to contain sufficient infectivity for a recipient to cross the 1% additional risk threshold (high risk plasma product), and (ii) it was considered likely that further implicated clotting factors would be identified if future clinical vCJD cases were found to have donated plasma.

Haemophilia clinicians used locally held or National Haemophilia Database (NHD) records to identify all recipients of UK-sourced plasma products from 1980 to 2001 and used product information from two UK fractionators to identify patients who had received implicated clotting factors. Patients notified as being 'at-risk' of vCJD for public health purposes were able to choose whether or not to find out if they had received implicated clotting factors. Haemophilia clinicians were encouraged to report these patients (unless they had withheld consent) to the NHD, for follow-up. This has been in the UKHCDO vCJD Surveillance Study following ethical approval from the London Multicentre Ethics Committee (MREC/01/2/11).

National haemophilia database

Data on product type and batch number of implicated batches, total doses received and start and completion dates of each treatment were collected by haemophilia centres. The NHD is updated annually by individual UK haemophilia centres with treatment data sets and information about new diagnoses and deaths. All deaths and causes of death are verified as patients are flagged with the Office for National Statistics. Person-years at risk of vCJD were calculated by subtracting the date of

the first dose of an implicated batch from either 1st January 2009 or the date of death as appropriate.

Results

Patient population in NHD

A total of 8547 patients with inherited bleeding disorders were registered on the NHD on 1st January 2009 (Table 1). Of these, 3735 have been identified as having received UK-sourced clotting factors between 1980 and 2001 and therefore are defined as 'at risk' of vCJD for public health purposes. Of these, 787 had received implicated clotting factors batches ('implicated batch') linked to donors who later developed clinical vCJD. Auditing notification data for each centre against implicated batches supplied to them by the two UK fractionators show that 11 million IUs (about 50%) of implicated batches remains unaccounted for [5]. As a result of this under-notification, it is estimated that the 787 patients represent approximately 50% of all patients who had received implicated batches. The following results/data concern these 787 implicated batch recipients.

Outcome, deaths and autopsy

No clinical cases of vCJD have been observed in these patients as of 1st January 2009. Fifty-one (6.5%) deaths were reported by 1st January 2009 but none was related to vCJD. Only four autopsies have been performed in this cohort. Abnormal prion protein, indicating vCJD infection, has been detected in a single postmortem spleen sample of a haemophilia patient who died of causes unrelated to vCJD 11 years after receiving 9025 IUs (estimated vCJD infectivity ID_{50} 0.21) from two implicated FVIII batches [7]. These batches were linked to two plasma donations from a donor who developed vCJD within 6 months of the second donation.

Estimated infectivity of implicated batches

Table 2 is the list of implicated batches showing the quantities of each batch used with their estimated infectivity, and the number of patients treated with each batch. Two hundred and sixty three (33%) patients received >1 implicated batches and 229 (29%) patients received implicated batches linked to >1 donors. A total of 12.7 million IUs of implicated FVIII and FIX was used to treat 787 patients from 1987 to 1999. On average each patient received 10 000 IUs (median) (range 240–169960) and estimated vCJD infectivity 0.443 ID_{50} (median) (range 0.010–9.593). A total of 773 (98%) patients received estimated vCJD infectivity ≥0.02 ID_{50} (Fig. 1). Of 604 (77%) patients who have been followed-up for over 13 years, which is the predicted incubation period of primary vCJD [10,11], 595 have ≥1%, 164 have ≥50% and 51 have 100% estimated vCJD risk in addition to the background UK population risks due to potential dietary exposure.

Donors linked to implicated batches

Table 3 is the list of eight donors showing data on implicated batches and the number of recipients linked to each of them. These donors developed vCJD 88 months (median) (range 6–143) following their last donations. One hundred and forty-nine (19%) patients received implicated batches linked to donors who developed vCJD within 6 months of donation and 552 (70%) linked to donors who developed vCJD within 6 years of donation. When estimated infectivity is plotted against interval between donation and onset of vCJD in donors the distribution of patients for these parameters can be clearly seen (Fig. 2). The patient in whom the abnormal prion protein associated with vCJD was found at postmortem received two implicated batches from donor 1 [7]. For one of these batches, the interval between donation and onset of vCJD in the donor is 6 months but with a relatively

Table 1. Patients with inherited bleeding disorders registered in the National Haemophilia Database on 1st January 2009 by diagnosis and subgroups at risk of vCJD for public health purposes.

	Number of patients with bleeding disorders by diagnostic subgroups				
Patient group and subgroups	Haemophilia A	Haemophilia B	von Willebrand	Other	Total
Total registered in the National Haemophilia Database (NHD)	3 281	729	2 996	1 541	8 547
Registered patients who are at risk of vCJD: (treated with UK sourced plasma products between 1980 and 2001)	2 246	562	518	409	3 735
Registered patients at risk of vCJD who are known to have received implicated clotting factor batches	556	168	39	24	787*

*11 million IUs (about 50%) of implicated batches remain unaccounted for [5]. As a result of this under-notification, it is estimated that the 787 patients represent approximately 50% of all patients who had received implicated batches.

Table 2. Description of each implicated clotting factor batch used to treat 787 patients with inherited bleeding disorders.

Batch number	Brand name[†]	Donor IDs linked to implicated clotting factor batches[*]	Months between donation and onset of vCJD in donors	Estimated infectivity (ID_{50}) per IU for each batch[†]	Total quantities (IUs) of each batch used	Estimated total infectivity (ID_{50}) received from each batch	Total number of patients registered as treated with each batch (n = 787)[‡]
FHB4547[§]	8Y	1	6	0.0000199	873 821	17.424	61
FHB4596	8Y	6	31	0.000043	1 054 410	45.340	93
FHC4237[§]	8Y	1	46	0.0000472	983 977	46.444	117
FHB4189	8Y	8	112	0.0000486	735 725	35.756	71
FHB4419	8Y	3	15	0.0000584	656 600	38.345	55
FHB4116	8Y	2	31	0.0000774	280 710	21.727	34
FHC0369	8Y	8	139	0.000088	199 060	17.517	52
FHC0289	8Y	2	59	0.0000948	266 960	25.308	46
FHC0059	8Y	5	143	0.0001135	58 560	6.645	10
FHM4054	High purity F8	8	127	0.0000662	304 500	20.158	33
FHM3990	High purity F8	8	134	0.0000738	169 055	12.476	11
FHE4548[§]	Replenate	1	6	0.0000246	965 400	23.749	88
FHF4625	Replenate	7	58	0.0000262	1 035 900	27.141	47
FHE4536	Replenate	6	40	0.000029	1 224 270	35.504	97
FHE4437	Replenate	8	82	0.0000388	818 095	31.742	73
0304-70510	Z8	4	134	0.0009526	16 150	15.385	3
FJA4308	9A	8	94	0.0000343	379540	13.031	20
FJA4239B[§]	9A	1	46	0.0000548	141435	7.755	9
FJA0092	9A	2	59	0.0000735	92990	6.835	18
FJA0020	9A	5	143	0.0000948	88025	8.349	10
3502-70210	HT Defix	4	138	0.0001391	216220	30.083	26
FJM4327	Replenine	8	98	0.0000226	1129915	25.536	80
FJM4625	Replenine	7	58	0.0000434	22145	0.961	4
FJM4437	Replenine	8	82	0.0000592	379380	22.459	29
FJM4596	Replenine	6	31	0.0000604	592380	35.780	49

[*]These numbers have been assigned to anonymize the donors for this study.
[†]Sorted by brand name and estimated infectivity per IU.
[‡]15 patients were treated with the same batch of an implicated clotting factor in more than one treatment episode. 256 patients were treated with different batches of implicated clotting factors in more than one treatment episode.
[§]Four implicated clotting factor batches were linked to the donor whose donations were linked to vCJD infection of a patient with bleeding disorder [7]. This patient received implicated clotting factor batches: FHB4547 and FHC4237.

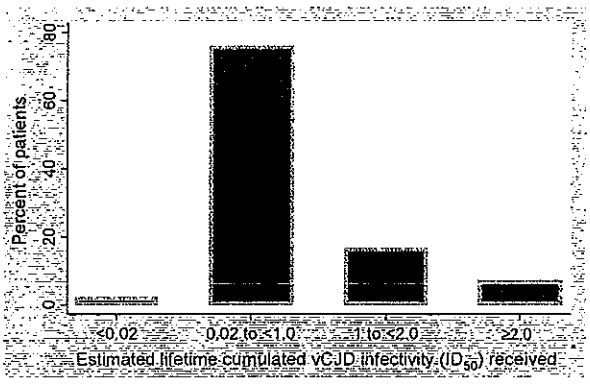

Fig. 1. Distribution of patients with bleeding disorders by estimated lifetime cumulated vCJD infectivity received (n = 787).

low estimated infectivity dose. Others have received higher estimated infectivity from the same donor and the same donation, but none of them has developed clinical vCJD.

Age at exposure and person-years at risk

The median age at which patients received their first dose of an implicated batch was 22 years (range 0.3–87). 174 (22%) patients were under 10 years, 362 (46%) under 20 years, and 628 (80%) were under 40 years of age when they received their first dose. The median age of patients who were alive on 1st-January-2009 (n = 736) was 35 years (range 13–92). The median follow-up time from the date of the first dose of an implicated batch to 1st January 2009 or the date of death was 15 years (range 2 days–22 years) (person-years at risk). Plotting the estimated infectivity against person-years at risk reveals many patients with more event free person-years at risk than the patient with known abnormal prion protein [7] (Fig. 3).

Discussion and conclusion

This article reports the absence of clinical vCJD cases among 787 patients with an inherited bleeding disorder who have been treated with high risk[2] implicated clotting factors. These include 604 (77%) patients who have lived longer after receiving the first doses of implicated clotting factors than the predicted incubation period of 13 years for primary vCJD [10,11]. Of them, one quarter (n = 164) have ≥50% estimated risk

[2]A single dose of implicated clotting factor was thought to contain sufficient infectivity for a recipient to cross the 1% risk threshold.

Table 3. Description of implicated donations, manufactured clotting factors, known quantities used, the number of identified patients treated, average quantities and infectivity received by each patient from individual donors.

Donor IDs	Months between donations and onset of vCJD in a donor*	Number of batches of each product linked to a donor (n = 25)		Total quantities (IUs) of implicated clotting factors linked to a donor	Total number of patients linked to a donor (n = 787)†	Average quantities (IUs) received by each patient (median, range, quartiles)	Average vCJD infectivity (ID_{50}) received by each patient (median, range, quartiles)
1‡	6,§ 46§	8Y	2	2 963 633	257	9000	0.245
		9A	1			260–96000	0.010–4.531
		REPLENATE	1			3000–15700	0.118–0.481
2‡	31, 59	8Y	2	640 660	83	5900	0.483
		9A	1			255–29820	0.024–2.527
						1770–11000	0.145–0.914
3	15	8Y	1	656 600	55	9400	0.549
						470–54990	0.027–3.211
						4700–16000	0.274–0.943
4‡	135, 138	HT DEFIX	1	232 370	29	5520	0.883
		Z8	1			552–48852	0.077–9.593
						2760–8800	0.384–1.690
5	143	8Y	1	146 585	20	4800	0.502
		9A	1			240–35645	0.027–3.381
						1928–9650	0.197–1.089
6‡	31, 40	8Y	1	2 871 060	238	10000	0.373
		REPLENATE	1			500–102960	0.015–3.851
		REPLENINE	1			4755–15300	0.155–0.616
7	58	REPLENATE	1	1 058 045	51	13510	0.472
		REPLENINE	1			965–57900	0.025–1.517
						5150–30880	0.152–0.809
8‡	82, 94, 98, 112,	8Y	2	4 112 770	336	9700	0.376
	127, 134, 139	9A	1			460–100000	0.011–5.511
		HPF VIII	2			3475–15343	0.178–0.660
		REPLENATE	1				
		REPLENINE	2				

*Median interval between donation and onset of vCJD in donors was 88 months. 149 (19%) of patients received implicated batches linked to donors who developed vCJD within 6 months of donation and 552 (70%) linked to donors who developed vCJD within 6 years of donation.
†The figures in the column do not add to 787 because of exposure to multiple-implicated donors. 557 patients were treated with implicated clotting factor batches linked to one donor, 182 to two donors, 45 to three donors, two to four donors, and one to five donors.
‡Donors 1, 2, 4, 6 and 8 donated more than once.
§The vCJD infected patient was treated with implicated clotting factor batches linked to two donations from this donor [7].

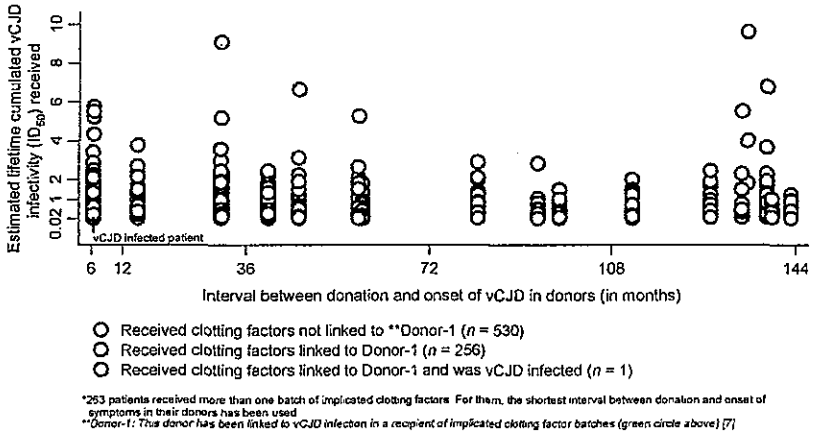

Fig. 2. Scatterplot showing estimated lifetime cumulated vCJD infectivity of implicated clotting factors received by patients with bleeding disorders by interval between donation and onset of symptoms in donors* (n = 787).

(received ≥1ID_{50}) and 8% (n = 51) have 100% risk (received ≥2ID_{50}) of vCJD in addition to background UK population risk due dietary exposure. Forty-nine of the 51 patients who have 100% risk were still alive on 1st January 2009. Thirteen of these 49 patients had received clotting factors linked to donors who developed vCJD within 6 months of their donation. The risk to these patients was calculated using estimates from the DNV risk assessment, and batch-manufacturing data.

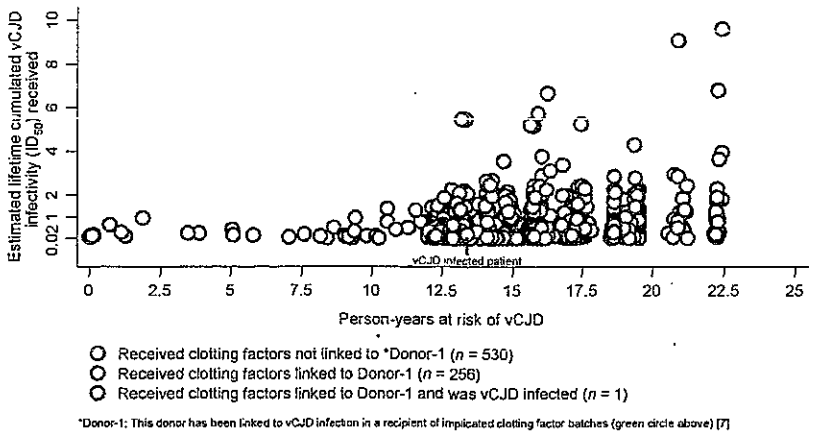

Fig. 3. Scatterplot showing estimated lifetime cumulated vCJD infectivity of implicated clotting factors received by patients with bleeding disorders by their person-years of exposure (n = 787).

The incubation period of vCJD within this at risk group may prove to be longer than the predicted incubation period of primary vCJD and secondary vCJD due to non-leucodepleted packed red cells transfusion. The infective dose in the plasma and red cell components is assumed equal but the implicated plasma is diluted in the plasma pool and then distributed between many vials. A large body of data from different experimental approaches (including endogenous infectivity models) consistently show that conventional bio-separation processes used in plasma product manufacturing are capable of removing prion agents to a significant extent [12,13]. These data question whether the highly precautionary approach as adopted in the UK is still judged as appropriate. It is possible that the infectivity clearance assumptions made in the DNV risk assessment, and the option chosen by the CJDIP are overly precautionary.

Other countries have adopted less precautionary approaches. Authorities in France concluded that the risk posed by implicated batches, even in the most pessimistic scenario, was very low. Consequently, they decided to continue to fractionate plasma sourced from domestic blood supply, introducing nano-filtration as an additional step in the process [14]. Authorities in Canada concluded that the risk of transmission of vCJD for patients who have received FXI linked to UK donors is in the range of 1 in 100 000 to 1 in 1 000 [15]. In their risk assessment, the US FDA included infectivity reductions associated with various processing steps in the production of FVIII and has concluded that the risk of vCJD infection is likely to be extremely low ranging from 1 in 9.4 million to 1 in 15 000 [16].

Age dependent susceptibility is required to fully account for observed age distribution of primary vCJD cases [11]. Age at treatment (8–10 years) with human growth hormone has been found to be a risk factor for secondary CJD in the UK [17]. If age dependent susceptibility is a risk factor for secondary vCJD, then the 174 (22%) patients who received their first dose of implicated clotting factors before 10 years of age may have an increased susceptibility to vCJD infection. The median follow-up time from first exposure in this subgroup is 16 years (range 12–22).

It is of interest that a recent publication links impaired scrapie agent neuroinvasion in aged mice with effects of host age on follicular dendritic cell status [18]. If immune function affects vCJD neuroinvasion in man, then it can be speculated that the immune modulation and deficiency associated with blood borne virus infections in some of this cohort may make subclinical vCJD infection more likely rather than clinical disease.

The dose response relationship has not been established for TSE infections. Experimental estimation of dose response relationship requires a large number of experimental animals, particularly if the level of infectivity is low. Unfortunately, there is very little data on dose response relationship in TSE infections. The DNV risk assessment considered different models on dose response relationship in TSE infections using available data and came up with the assumption that the dose-response function for vCJD infectivity is linear without any threshold [6]. More experimental data are required to validate this assumption to improve the risk assessment.

The DNV risk assessment assumes that risk from regular equal doses of vCJD implicated plasma product over a 1-year-period is additive, and it ignores doses received after the first year. Where the patients have received variable doses from different batches and/or from different donors during several years with wide variations in the estimated levels of infectivity, it is difficult, and somewhat meaningless to calculate an annual dose. Therefore, the CJDIP took a precautionary approach and decided to estimate cumulative lifetime infectivity.

While the under reporting of implicated batch recipients is a concern, it does not invalidate the descriptive data on risk assessment. These may inform any future risk assessment should vCJD develop in a patient who has received implicated batches of clotting factors.

Other factors, such as, prion protein genotype, age at exposure, interval between donation and development of vCJD in the donor, lifetime cumulative infectivity received and the number of donor exposures may also help assess the vCJD risk in this cohort. The continuance of this surveillance study especially with improved recruitment to its postmortem and biopsy arm may provide valuable information that aids our understanding of developing vCJD after exposure to implicated clotting factor batches and allows more informed risk counselling of patients.

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Disclosures

The authors stated that they had no interests which might be perceived as posing a conflict or bias.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Users Guide to the Product Risk Calculator.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

B　個別症例報告概要

○　総括一覧表
○　報告リスト

個別症例報告のまとめ方について

個別症例報告が添付されているもののうち、個別症例報告の重複を除いたものを一覧表の後に添付した（国内症例については、資料３において集積報告を行っているため、添付していない）。

別紙様式第4

感染症発生症例一覧

	番号		感染症の種類		発現国	性別	年齢（歳）	発現時期（年/月/日）	転帰	出典	区分	識別番号	報告日	備考 MedDRA (Ver.)
			器官別大分類	基本語										
第17回	17	-1	感染症および寄生虫症	C型肝炎	米国	女性	不明	1993	不明	自発報告	当該製品	11065985	2011/7/12	14.0
第17回	17	-2	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	死亡	自発報告	当該製品	11000014	2011/8/31	14.0
第17回	17	-3	感染症および寄生虫症	C型肝炎	フランス	男性	不明	不明	不明	自発報告	当該製品	11000015	2011/9/1	14.0
第17回	17	-4	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000016	2011/9/1	14.0
第17回	17	-5	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000017	2011/9/1	14.0
第17回	17	-6	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000018	2011/9/1	14.0
第17回	17	-7	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000019	2011/9/1	14.0
第17回	17	-8	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000020	2011/9/1	14.0
第17回	17	-9	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000021	2011/9/1	14.0
第17回	17	-10	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000022	2011/9/1	14.0
第17回	17	-11	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000023	2011/9/1	14.0
第17回	17	-12	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000024	2011/9/1	14.0
第17回	17	-13	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000025	2011/9/1	14.0
第17回	17	-14	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000026	2011/9/1	14.0
第17回	17	-15	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000027	2011/9/1	14.0
第17回	17	-16	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000028	2011/9/1	14.0
第17回	17	-17	感染症および寄生虫症	C型肝炎	スペイン	不明	不明	不明	不明	自発報告	当該製品	11000029	2011/9/1	14.0
第17回	17	-18	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000030	2011/9/1	14.0
第17回	17	-19	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000031	2011/9/1	14.0
第17回	17	-20	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000032	2011/9/1	14.0
第17回	17	-21	感染症および寄生虫症	C型肝炎	スペイン	女性	不明	不明	不明	自発報告	当該製品	11000033	2011/9/1	14.0
第17回	17	-22	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000034	2011/9/1	14.0
第17回	17	-23	感染症および寄生虫症	C型肝炎	スペイン	男性	不明	不明	不明	自発報告	当該製品	11000035	2011/9/1	14.0
第17回	17	-24	臨床検査	B型肝炎コア抗体陽性	米国	不明	不明	不明	不明	自発報告	外国製品	11103879	2011/9/15, 2011/9/28	14.0
第17回	17	-25	感染症および寄生虫症	C型肝炎	米国	女性	不明	不明	不明	自発報告	外国製品	11000040	2011/11/10	14.1
第17回	17	-26	感染症および寄生虫症	C型肝炎	米国	男性	不明	不明	不明	自発報告	外国製品	11000043	2011/11/17	14.1

血対ID	受理日	番号	報告者名	一般名	生物由来成分名	原材料名	原産国	含有区分	文献	症例	適正措置報告
100473	27-Dec-11	110791	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人免疫グロブリンG	人血漿	米国	有効成分	無	有	無
100474	27-Dec-11	110792	バクスター	乾燥イオン交換樹脂処理人免疫グロブリン	人血清アルブミン	人血漿	米国	添加物	無	有	無

別紙様式第４

感染症発生症例一覧

	番号	感染症の種類		発現国	性別	年齢	発現時期（年/月/日）	転帰	出典	区分	備考
		器官別大分類	基本語								
第17回	17-1	感染症および寄生虫症	Ｃ型肝炎	コロンビア	男	８歳	不明	不明	症例報告	当該製品	識別番号：11000011 報告日：2011年8月22日 MedDRA: Version(14.0)
第16回	16-1	感染症および寄生虫症	非Ａ非Ｂ型肝炎	アルゼンチン	男	46歳	不明	回復	文献報告	当該製品	識別番号：11000001 報告日：2011年4月15日 MedDRA: Version(14.0)

100476	20-Jan-12	110803	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ルリオクトコグアルファ(遺伝子組換え)	遺伝子組換えチャイニーズハムスター卵巣細胞株	該当なし	有効成分	無	有	無
100477	20-Jan-12	110804	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ウシ血清アルブミン	ウシ血液	米国	製造工程	無	有	無
100478	20-Jan-12	110805	バクスター	ルリオクトコグアルファ(遺伝子組換え)	インスリン(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ膵臓	米国	製造工程	無	有	無
100479	20-Jan-12	110806	バクスター	ルリオクトコグアルファ(遺伝子組換え)	アプロチニン	ウシ肺	ニュージーランド	製造工程	無	有	無
100480	20-Jan-12	110807	バクスター	ルリオクトコグアルファ(遺伝子組換え)	ウシ胎児血清(抗第Ⅷ因子モノクローナル抗体製造用)	ウシ血液	オーストラリア	製造工程	無	有	無
100481	20-Jan-12	110808	バクスター	ルリオクトコグアルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-1)	ウシ血液	米国	製造工程	無	有	無
100482	20-Jan-12	110809	バクスター	ルリオクトコグアルファ(遺伝子組換え)	培養補助剤(抗第Ⅷ因子モノクローナル抗体製造用-2)	ウシ肝臓	米国又はカナダ	製造工程	無	有	無
100483	20-Jan-12	110810	バクスター	ルリオクトコグアルファ(遺伝子組換え)	人血清アルブミン	人血漿	米国	添加物	無	有	無