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。

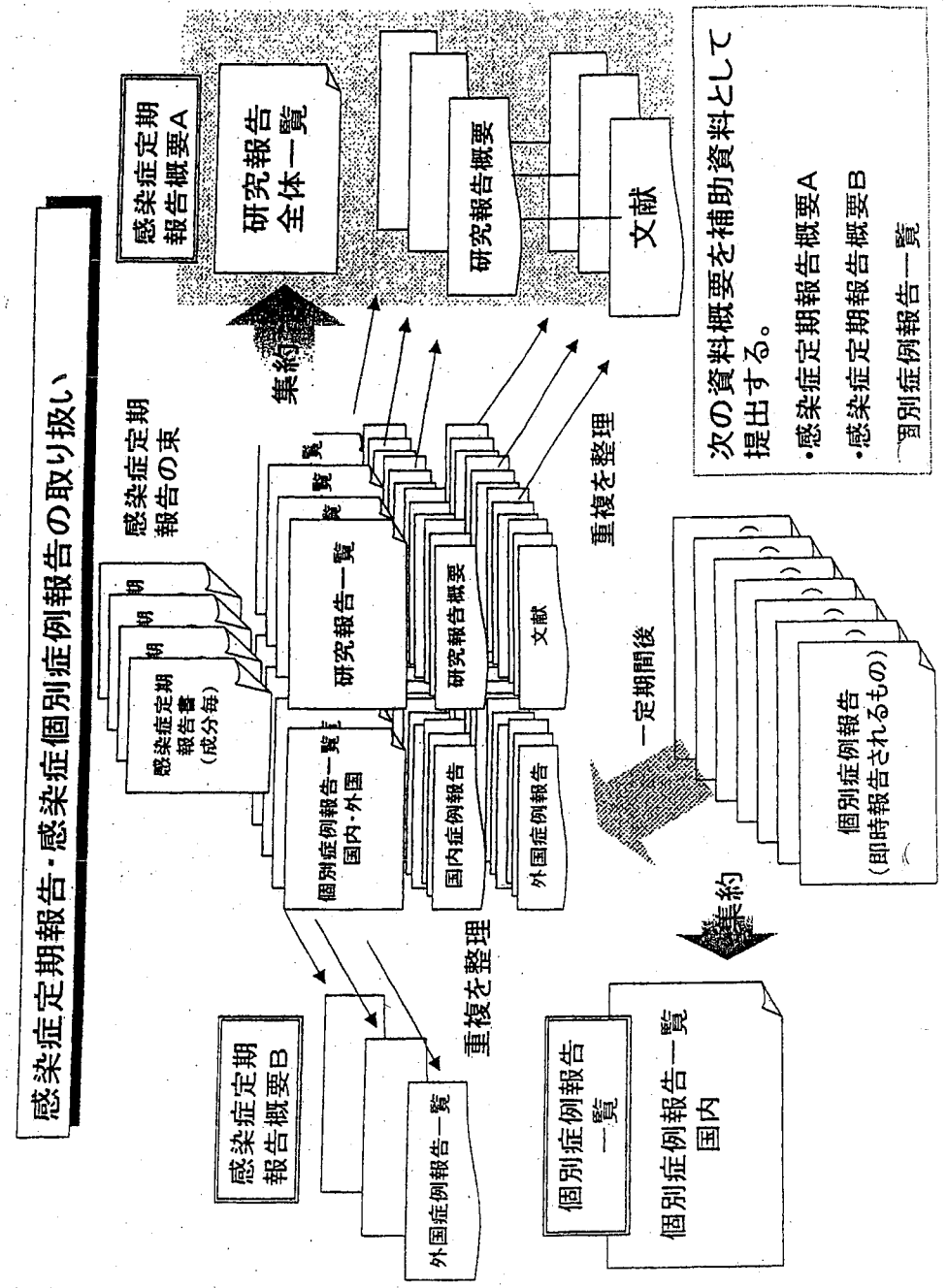
① 研究報告は、同一文献による重複を廃した別紙のような形式の一覧表を作成し、当該一覧表に代表的なものの報告様式（別紙様式第2）及び該当文献を添付した「資料概要A」を事務局が作成し、送付する。

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。



感染症定期報告概要

（平成２１年５月１４日）

平成２０年１２月１日受理分以降

Ａ　研究報告概要
Ｂ　個別症例報告概要

Ａ　研究報告概要

○　一覧表（感染症種類毎）
○　感染症毎の主要研究報告概要
○　研究報告写

研究報告のまとめ方について

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

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

感染症定期報告の報告状況(2008/12/1～2009/2/28)

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

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90064	2008/12/01	80762	B型肝炎C型肝炎	第56回日本輸血・細胞治療学会総会 2008年4月25-27日 P-033	2007年に医療機関から日本赤十字社に報告された輸血関連感染症の報告数は124例(10月末現在)であり、一昨年及び昨年の同期間に比べ減少傾向にある。内訳はHBVが61例、HCV32例、細菌24例、その他のウイルスが7例であった。ウイルス感染(疑)症例の調査結果により病原体を確認した症例は、HBVの12例とHCVの1例であった。HCVの1例は20プールNAT開始後(2004年8月開始)初めての検出限界以下の献血血液による感染症例であった。	
90072	2008/12/17	80788	C型肝炎	第70回 日本血液学会総会 2008年10月10-12日	症例は再生不良性貧血の54歳の女性で、2007年6月20日に初回輸血が実施され、初回輸血前検査はHCV抗体陰性、HCVコア蛋白陰性で、あった。10月1日の輸血後、HCVコア蛋白が陽性化したため、遡及調査を開始した。患者には計54本の輸血があり、保管検体の個別NATにより、1検体からHCV-RNAを検出した。患者と献血者のHCV Core-E1-E2領域の塩基配列が一致したことから、本症例は輸血によるHCV感染である可能性が極めて高い。	3
90064	2008/12/01	80762	C型肝炎	Clin Infect Dis 2008; 47: 627-633	フランスの大学病院の血液透析ユニットでのHCV伝播リスクにおける環境汚染および標準的注意の非遵守の役割を評価した。試験期間中にHCV陽性となった2名のうち1名は、同ユニットで治療中の慢性感染患者と同じウイルス株に感染していることが系統遺伝学的解析により明らかとなった。環境表面検体740例中82例がヘモグロビンを含み、その内6例がHCV RNAを含んでいた。手の衛生に関する遵守率は37%、患者ケアの直後に手袋をはずしていたのは33%であった。	
90064	2008/12/01	80762	C型肝炎	Clin Infect Dis 2008; 47: 931-934	ニューヨーク市のEast Harlemのクリニックから18歳以上で血中HCV PCR陽性の吸引用麻薬常習者38名の鼻汁検体および吸引に使用したストローを入手し、血液およびHCV RNAの存在の有無を調べた。鼻汁検体28例(74%)、ストロー3例(8%)から血液が検出され、鼻汁検体5例(13%)、ストロー2例(5%)でHCV RNAが検出された。HCVウイルスの鼻腔内伝播のウイルス学的妥当性が示された。	4
90064	2008/12/01	80762	E型肝炎	Am J Trop Med Hyg 2008; 78: 1012-1015	スペインでブタに曝露しているヒト101名と曝露していないヒト97名におけるHEV感染の有無を調べた。抗HEV IgG保有率は曝露群では18.8%、非曝露群では4.1%であった。ブタに接するヒトの抗HEV IgG保有リスクは5.4倍(P=0.03)であった。HEV感染は養豚作業員の職業病として扱うべきである。	
90078	2009/01/26	80844	E型肝炎	Transfusion 2008; 48: 1368-1375	2004年9月20日に39歳日本人男性から献血された血液はALT高値のため不適当とされ、HEV陽性であった。当該ドナーの遡及調査の結果、9月6日にも献血を行い、HEV RNAを含有する血小板が輸血されていた。当該ドナーと親戚は8月14日にブタの焼肉を食べており、父親は9月14日に急性肝炎を発症し、E型劇症肝炎で死亡した。他に7名がHEV陽性であった。レシピエントは輸血22日目にALTが上昇し、HEVが検出された。	
90075	2009/01/09	80834	E型肝炎	Vox Sanguinis 2008; 95(Suppl.1): 282-283	2005年の中国の4都市(Beijing、Urmuchi、Kunmingおよび Guangzhou)における供血検体のHEV感染率を調べた。その結果、ルーチン検査(抗HCV、抗HIV1/2、HBsAg、梅毒およびALT)陰性供血者の約1%は抗HEV IgMまたはHEV Ag陽性で、HEV感染の可能性があった。また、ALTスクリーニングは中国のHEV感染血排除に役立つ可能性があった。	

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

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90064	2008/12/01	80762	ウイルス感染	PLoS Pathogens 2008; 4: e1000047	出血熱症例の小さな流行が、2003年12月と2004年1月にボリビアのCochabamba付近で発生した。1死亡例から検体を入手し、患者血清検体から非細胞障害性ウイルスを単離し、アレナウイルスと同定した。RT-PCR分析、並びにS及びL RNAセグメント配列の解析の結果、このウイルスはサビアウイルスに最も近縁であるが、新規のウイルスであることが示された。我々はこのウイルスをChapareウイルスと命名することを提案する。	
90066	2008/12/16	80781	ウイルス感染	Proc Natl Acad Sci USA 2008; 105: 14124-14129	インフルエンザ様疾患の小児の呼吸分泌物中から、汎ウイルスマイクロアレイ法を用いて、初めてヒトカルディオウイルスを同定した。系統遺伝学的分析から、このウイルスはTheilerのネズミ脳脊髄炎ウイルス亜型に属し、SaffoldウイルスAと最も近縁であった。また、胃腸疾患患者群498名から得た751例の糞便検体中6検体からカルディオウイルスが検出された。	
90064	2008/12/01	80762	ウイルス感染	ProMED-mail20080720.2201	オーストラリアBrisbaneの動物病院のスタッフが数死性のヘンドラウイルスに感染した。看護師1名と獣医1名が、感染したウマ数頭を治療後、感染した。前回のアウトブレイクは1994年で調教師1名とウマ14頭が死亡した。同ウイルスがヒト-ヒト感染するとのエビデンスはなく、拡大する危険性はない。	
90066	2008/12/16	80781	ウイルス感染	ProMED-mail20081028.3409	2008年10月初旬に南アフリカでアレナウイルスによる感染のアウトブレイクが同定された。9月12日から10月24日までに計5例が報告され、5例中4例が死亡し、1例は入院中である。死亡した4例では発病から死亡まで9～12日間であった。塩基配列分析より、ユニークな旧世界アレナウイルスが原因であることが明らかとなった。現在のところ新たな疑い症例はない。	8
90075	2009/01/09	80834	ウイルス感染	Transfusion 2008; 48: 1180-1187	米国テキサス南東部の健康な成人ドナー100名の血液中のヒトヘルペスウイルス(HHV)陽性率とウイルスDNA量をRT-PCRにより調べた。その結果、HSV-1、HSV-2、VZV及びHHV-8 DNAはどの検体からも検出されなかった。一方、EBVは72%、HHV-7は65%、HHV-6は30%、CMVは1%に検出された。また、1名の血液から6.1×10^7geq/mlを超えるHHV-6 Type Bが検出されたが、健常者における異常な高値は活動性感染や免疫不全とは関連が無いと思われる。	
90075	2009/01/09	80834	ウイルス感染	WHO/EPR 2008年10月13日	南アフリカとザンビア出身者の最近の死亡例3例はアレナウイルス科のウイルスが原因あることが、NICDおよびCDCで行われた検査の結果明らかとなった。このウイルスに関する詳細な分析が継続されている。一方、南アフリカでは患者と密接に接触した看護師が感染し、入院中である。	
90066	2008/12/16	80781	ウイルス性脳炎	ProMED-mail20080828.2697	インド東部のウッタルプラデシ州で小児を死亡させている原因不明のウイルスは、インド保健省の専門家らにより急性脳炎症候群と診断された。同州の13の地区では、数週間におよそ800人の患者が発生し150人が死亡したと報告され、その数は増加すると見られている。血液検査で日本脳炎陽性となった患者は5%以下であった。日本脳炎とエンテロウイルスとの混合感染の可能性について調査中である。	
90068	2008/12/17	80784	ウエストナイルウイルス	ABC Newsletter No.38 2008年10月17日	2008年9月に、イタリアで何年かぶりにヒトのウエストナイルウイルス(WNV)脳炎が2例報告された。1例目はFerraraとBolognaの間に住む80歳代の女性、2例目はFerraraに住む60代後半の男性であった。また、ウマ6頭とトリ13羽でWNV感染が確認された。WNV髄膜脳炎の積極的サーベイランスプログラムが開始され、当該地域で供血者スクリーニング用NATが導入された。また、当該地域に1日以上滞在したことのある供血者を28日間供血延期する措置がとられた。	9

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90064	2008/12/01	80762	ウエストナイルウイルス	Rev Panam Salud Publica 2006; 19: 112-117	文献および未発表データから、ラテンアメリカやカリブ海地域のウエストナイルウイルス(WNV)感染の現状をまとめた。WNV感染は2001年にCayman諸島とFlorida Keysの住民で見られ、2002～2004年にジャマイカ、メキシコなど周辺地域で動物や鳥類での感染が確認されている。しかし、疾患報告数は少ない。この不可解な熱帯生態系でのウイルス減弱または他の可能性を検討するためには分離株が必要である。	
90068	2008/12/17	80784	クロイツフェルト・ヤコブ病	J Neurol Neurosurg Psychiatry 2008; 79: 229-231	オーストリアの39歳男性が感覚異常などの神経症状で入院後、急速に悪化し、4ヶ月後に死亡した。組織学的検査で海綿状変化、神経細胞脱落及びグリオーシスが、免疫組織化学的検査でびまん性シナプティックな異常プリオンの沈着が見られ、CJDと診断された。また患者のPRNPは129Met-Metであった。患者は22年前まで死体由来のヒト成長ホルモン(hGH)製剤治療を受けており、医原性リスクが認められるため、孤発性若年性CJDの可能性も否定できないが、WHO基準により確定医原性CJDと分類された。	10
90064	2008/12/01	80762	コンゴ・クリミア出血熱	ProMED-mail20080709.2092	2008年7月7日、トルコのBursa、CanakkaleおよびSamsunの病院でダニ媒介性疾患であるクリミア・コンゴ出血熱により3名が死亡し、この2ヶ月での死者数は37名となった。保健省はダニに注意するよう呼びかけ、咬まれた場合は決して手でつぶさずに、医師にピンセットで注意深く取り除いてもらい、ヨードで消毒することを推奨している。	
90064	2008/12/01	80762	サルモネラ	CDC 2008年7月8日	CDCは関係機関と協力して複数の州で発生したサルモネラ血清型セントポールのアウトブレイクを調査している。生のトマトの摂食が原因と考えられている。2008年4月以降2008年7月7日までに、米国の41の州、ワシントンD.C.およびカナダで991名の患者が同じ遺伝子パターンのサルモネラ血清型セントポールに感染したことが確認された。	
90075	2009/01/09	80834	チクングニヤウイルス感染	Transfusion 2008; 48: 1333-1341	2005年から2007年に、チクングニヤウィルス(CHIKV)はレユニオン島で大流行し、供血は2006年1月に中断された。大流行中のウイルス血症血供血の平均リスクは、10万供血あたり132と推定された。2006年2月の最流行時におけるリスクは、10万供血あたり1500と最高であった。この期間中、757000人の住民のうち推定312500人が感染した。2006年1月から5月の平均推定リスク(0.7%)は、CHIKV NAT検査による血小板供血のリスク(0.4%)と同じオーダーであった。	
90064	2008/12/01	80762	デング熱	Hong Kong Med J 2008; 14: 170-177	1998～2005年に香港の公立病院に入院したデング確定患者全員の医療記録をレトロスペクティブに検討した。126例中123例(98%)がデング熱、3例(2%)がデング出血熱であった。1例が輸血により感染したデング熱であった。116例が輸入症例、10例が地域症例であった。デングウイルス1型が最も多く、次に2型、3型、4型の順であった。死亡例はなかった。発熱、皮疹を呈し、血小板減少などを示す渡航歴のある患者には鑑別診断にデング熱を含めるべきである。	
90075	2009/01/09	80834	デング熱	Transfusion 2008; 48: 1342-1347	高力価の培養デングウイルス　セロタイプ2をアルブミンおよび免疫グロブリンの各種製造工程(低温エタノール分画、陽イオン交換クロマトグラフィー、低温殺菌、S/D処理およびウイルスろ過)前の検体に加え、各工程での同ウイルスのクリアランスをVero E6細胞培養におけるTCID50アッセイおよびRT-PCRで測定した。その結果、全ての工程が不活化・除去に有効であることが示された。	
90075	2009/01/09	80834	デング熱	Transfusion 2008; 48: 1348-1354	2005年9月20日～12月4日のプエルトルコの米国赤十字におけるすべての供血16521検体中のデングウイルス(DENV) RNAをTMA(transcription-mediated amplification)法で測定したところ、12検体(0.07%)がTMA陽性であった。4検体は、RT-PCR(DENVセロタイプ2および3)陽性であった。RT-PCR陽性4検体中3検体でウイルスを培養することができた。TMA陽性12検体中1検体がIgM陽性であった。1:16に希釈した場合は5検体のみTMA陽性であった。	

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90064	2008/12/01	80762	バベシア症	American Society for Microbiology 108th General Meeting 2008年6月1-5日、Boston	米国中南部では稀な輸血によると考えられるBabesia microti感染症例の報告である。61歳の女性患者で、赤血球輸血後、吐き気と発熱を訴え、敗血症の症状を呈し、死亡した。血液塗抹標本で赤血球の5～15%にトロフォゾイト(栄養体)があった。患者血液検体中でBabesiaは形態学的に確認され、PCRでB. microti陽性であった。輸血された製剤の供血者のうち1名がB. microti陽性であった。	
90075	2009/01/09	80834	パルボウィルス	FDA/CBER 2008年7月　業界向けガイダンス(案)	血漿由来製品によるパルボウイルスB19伝播リスクを低減するための核酸増幅検査(NAT)についてのガイダンス案が示された。全ての血漿由来製剤について、製造プール中のパルボウイルスB19 DNAのウイルス負荷を確実に10000 IU/ml未満とするため、製造過程の品質管理検査としてNATを実施すべきである。ミニプール中でのNATの感度は少なくとも1000000 IU/mlとするべきである。これらの基準を超えるものは使用してはならない。	
90078	2009/01/26	80844	パルボウィルス	Lab Hematol. 2007; 13: 34-38	血漿交換、コルチコステロイドおよびコリンエステラーゼ阻害剤による治療を受けていた重症筋無力症患者が、アルブミンを用いた血漿交換を行った2週後にパルボウイルスB19感染による赤芽球減少症と診断された。アルブミン由来感染かどうかを確定することはできなかったが、アルブミンなどの血液製剤によるB19感染を除外することはできない。	11
90064	2008/12/01	80762	パルボウィルス	Transfusion 2008; 48: 1036-1037	大阪における1997-1999年の献血者979052名中102名がヒトパルボウイルスB19感染者であった。B19感染者のうち20名のB19 DNA、IgGおよびIgMを長期間フォローアップしたところ、B19持続感染が観察されたが、B19感染の症状を報告した者はいなかった。B19急性感染後の血漿ウイルス力価は約1年で10^4IU/mL未満、約2年で10^3IU/mL未満まで下がることが示された。	
90064	2008/12/01	80762	ハンタウイルス	Emerg Infect Dis 2008; 14: 808-810	スウェーデンにおけるPuumalaウイルスの予期せぬ大規模アウトブレイクにより、2007年のVästerbotten地方の流行性腎症患者の数は100,000人当り313人に至った。齧歯類の増加の他、気候温暖化および地表を覆う積雪の減少により、ウイルスを媒介するハタネズミの活動が活発だったことが、当該アウトブレイクの一因であろうと考えられる。	
90064	2008/12/01	80762	ブルセラ症	Clin Infect Dis 2008; 46: e131-136	急性ブルセラ症患者39名の血液検体中のBrucella DNAの存在をRT PCR法により調べた。その結果、治療終了時では87%、治療完了後6ヶ月では77%、治療後2年を過ぎても70%の患者で、無症候性であるにもかかわらず、Brucella DNAが検出された。適切な治療を行い、回復したように見えても、Brucella DNAは存続する。ブルセラ菌は除去不可能な持続性の病原体である。	
90064	2008/12/01	80762	マラリア	Emerg Infect Dis 2008; 14: 1434-1436	2007年にマレー半島でフィンランドの旅行者が、通常はサルにおけるマラリアの原因となる二日熱マラリア原虫に感染した。二日熱マラリア原虫はヒトマラリアを引き起こす第5のマラリア原虫種として確立された。この疾病は生命を脅かす危険があり、臨床医と臨床検査技師は旅行者においてこの病原体を更に注意すべきである。	12
90064	2008/12/01	80762	リケッチア症	Emerg Infect Dis 2008; 14: 1019-1023	ネコノミが媒介するRickettsia felis感染症のヒト症例は世界中で報告されている。症状は発疹熱やデング熱などに類似しており、実際よりも少なく推定されている可能性が高い。ヒトの健康を脅かす感染症として今後調査が必要である。	
90066	2008/12/16	80781	リケッチア症	ProMED-mail20080728.2306	オランダ・ブラバント州の公衆衛生局が行った調査でQ熱の症例報告数が急激に増加し、2008年7月21日付けで491症例が報告されている。感染症管理センター長によると、実際の感染者数は報告された症例数の10倍であると思われる。2007年まではQ熱はオランダではほとんど存在しなかった。	

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90066	2008/12/16	80781	レプトスピラ症	Infect Genet Evol 2008; 8: 529-533	コスタリカにおいて、レプトスピラ症の入院患者から分離されたレプトスピラは、Javanica血清群型に分類される新しい血清型で、Arenalと命名された。同じ地区の重症患者から分離された株も同じ血清型であったことから、この株は、この地域に流行する新規の高病原性の血清型であると考えられた。	
90064	2008/12/01	80762	異型クロイツフェルト・ヤコブ病	2008年プリオン研究会 2008年8月29-30日	CJDサーベイランス委員会による調査では1999年4月から2008年2月までの9年間に日本国内で1069例がプリオン病と判定された。うち孤発性CJDが821例(76.8%)、遺伝性プリオン病が171例(16.0%)、硬膜移植後CJD74例(6.9%)、変異型CJD1例(0.1%)、分類不能2例(0.2%)であった。日本のプリオン病剖検率は欧米諸国より著明に低かった。孤発性CJDの病型は欧米に比べMM2型が多かったが、非典型例が多く剖検されている可能性が考えられた。	13
90064	2008/12/01	80762	異型クロイツフェルト・ヤコブ病	2008年プリオン研究会 2008年8月29-30日 ポスター11	ウイルス除去膜濾過工程を含んでいる製剤(血液凝固第VIII因子製剤:プラノバ20N濾過、抗HBs人免疫グロブリン製剤:プラノバ35N濾過)について、263K株感染ハムスターより得たSUS処理PrPScを用いて、その除去効果を検証した。その結果、SUS処理PrPScは濾過膜の孔径よりも小さいにもかかわらず、プラノバ35Nやプラノバ20Nで除去された。PrPScが凝集したり、膜へ吸着したためと考えられる。	14
90064	2008/12/01	80762	異型クロイツフェルト・ヤコブ病	2008年プリオン研究会 2008年8月29-30日 ポスター18	スクレイピー263K感染ハムスター脳乳剤を脳内接種したハムスターにおける血中PrPres経時的変化を追跡したところ、PK抵抗性3F4反応性蛋白バンドは、感染後4～6週で認められ、10週ではほぼ消失した。発症末期では血中PrPresと見られる蛋白バンドは認められなかった。PrPresをマーカーとした血液検査は感染後発症前～発症中期までに限定される可能性が示唆された。	15
90075	2009/01/09	80834	異型クロイツフェルト・ヤコブ病	American Society of Hematology/Press Releases 2008年8月28日	Blood誌のprepublished onlineに掲載されたヒツジにおける研究によると、輸血によるBSE伝播のリスクは驚くほど高い。エジンバラ大学で行われた9年間の研究は、BSEまたはスクレイピーに感染したヒツジからの輸血による疾病伝播率を比較した。その結果、BSEおよびスクレイピーとも輸血によりヒツジに効率よく伝播された。症状を呈する前のドナーから採取された血液によっても伝播することが示された。	
90064	2008/12/01	80762	異型クロイツフェルト・ヤコブ病	Ann Neurol 2008; 63: 697-708	米国の国立プリオン病病因調査センターの患者11名(平均発症年齢62歳)を調べたところ、海綿状変性の型、PrP免疫染色パターンおよびマイクロプラークの存在が、既知のプリオン病とは異なり、通常の方法では典型的なプロテアーゼ抵抗性PrPは検出されなかった。我々はこれらをプロテアーゼ感受性プリオン病(PSPr)と名付けた。PSPrは、プリオン病の中では稀ではなく、我々のデータが示すよりもさらに多い可能性がある。	
90064	2008/12/01	80762	異型クロイツフェルト・ヤコブ病	Blood, Prepublished online 2008年7月22日	ヒツジを用いた感染実験において、BSEは36%、スクレイピーは43%と予想以上に高い輸血伝播率を示した。高い伝播率および臨床的に陽性のレシピエントにおける比較的短期間の一定した潜伏期間は、血中の感染性力価が高いことおよびTSEが輸血により効率的に伝播することを示唆する。血液製剤によるヒトでのvCJD伝播を研究するために、ヒツジが有用なモデルであることが示された。	16

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

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90064	2008/12/01	80762	感染	Vox Sanguinis 2008; 95(Suppl. 1), 2A-S01-02	化学的または光化学的遺伝子修飾に基づいた血液製剤中の病原体不活化(PI)は広範囲のスペクトルの予防的アプローチである。溶媒界面活性剤(SD)およびメチレンブルー法は欧州の多くの国で使われている。アモトサレン(Intercept)、リボフラビンを用いた新しい方法が導入されている。リボフラビン、UVおよび可視光線を用いる血小板(PC)、血漿および赤血球のためのPI法が開発中である。	
90075	2009/01/09	80834	狂犬病	ProMED-mail20080826.2660	1990年から2007年の中国における狂犬病発生傾向を調べた研究によると、最近8年間でヒト狂犬病症例数が急激に増加したことが明らかとなった。ヒト狂犬病は1990年から1996年の間は全国的な狂犬病ワクチン接種プログラムにより抑制され、わずか159症例が報告されただけであるが、2006年は3279症例と激増した。	
90064	2008/12/01	80762	原虫感染	Emerg Infect Dis 2008; 14: 1013-1018	リーシュマニア症は生物媒介性疾患で、南ヨーロッパに定着しており、毎年700例近く、トルコを含めると3950例のヒトでの感染が報告されている。無症候症例は臨床症例の30～100倍とみられ、また飼い犬の血清陽性率は25%と推定される。薬剤耐性Leishmania infantumがイヌを介して拡大するおそれもある。全ヨーロッパレベルでの研究が必要である。	
90068	2008/12/17	80784	細菌感染	Am J Infect Control 2008; 36: 602	減量法として両耳の上部耳介軟骨に重ね鋲治療(Stapling)を受けた18歳の女性が、2週間後に左耳の鋲周囲の紅斑および圧痛を呈した。膿瘍ドレナージ検体の培養および感受性試験の結果、両耳で著しい緑膿菌の生育が認められた。21日間の経口シプロフロキサシン投与により回復した。外耳軟骨は、血流に乏しく特に感染しやすい。耳鋲が危険な緑膿菌感染を起こす可能性があることを医師は認識するべきである。	17
90072	2008/12/17	80788	細菌感染	American Society for Microbiology 108th General Meeting 2008 年6月1-5日	マサチューセッツの医療センターで品質管理のため使用された廃棄製剤、使用期限切れロット、アフェレーシスの残りの人血清アルブミン製剤を入手し、クラミジアの有無を調べた。その結果、PCR及びウエスタンブロットにより、4社の20製剤全てにおいてクラミジアの存在が確認された。また、in vitro培養を行ったところ11検体(55%)でクラミジア生菌が生育した。	
90066	2008/12/16	80781	細菌感染	CDC/MMWR 2008; 57: 1145-1148	米国ミネソタ州の68歳男性が、2007年10月12～21日に手術後の輸血を受け、敗血症および多臓器不全をきたした後、10月31日に発熱を伴う急性血小板減少症を発現し、11月3～5日の血液検体からPCR及び抗体検査でアナプラズマ症感染が確認された。血液ドナーの1人にA. phagocytophilum陽性がPCR及びIFA検査で確認され、血液ドナーに感染源が確認された初の事例となった。	18
90064	2008/12/01	80762	細菌感染	Transfusion 2008; 48: 1520-1521	骨髄異形成症候群と汎血球減少症の79歳男性が、血小板輸血と続いて赤血球1単位の輸血を受けた。 40分後に39.6℃Cの発熱、硬直、背部痛、低血圧および低酸素症を呈し、輸血は中止された。患者は抗菌剤による治療で回復した。患者の血液および赤血球バッグの残存物からStreptococcus pneumoniae血清型4が検出された。赤血球輸血によるS pneumoniae感染の初めての症例である。	
90064	2008/12/01	80762	細菌感染	第56回 日本輸血・細胞治療学会総会 2008年4月25-27日 WS-3-3	血小板濃厚液の輸血後に、TRALI様の急性呼吸不全と髄膜炎を併発し、血小板残液からBacillus cereusが検出された症例の報告である。TRALI様の急性呼吸不全を呈した際は、輸血後感染症も視野に入れた対応が必要である。髄膜炎併発例の報告はこれまでに無いが、輸血後感染症治療では髄液移行性も考慮した抗生剤選択が求められる。培養検査だけでなく、遺伝子検査まで施行することが、診断及び同一菌株の証明に重要である。	

血対ID	受理日	番号	感染症(PT)	出典	概要	新出文献No.
90077	2009/01/21	80839	鳥インフルエンザ	Proc Natl Acad Sci USA 2008; 105: 7558-7563	ユーラシアおよび北米系統のH7型トリインフルエンザウイルスの受容体結合能およびフェレットモデルにおける感染性を調べた。その結果、2004年にカナダで分離されたH7N3型、2002-2003年に米国北東部で分離されたH7N2型はα2-6結合シアル酸に対する親和性を高めたHAを保有していた。また2003年にニューヨークの男性から分離された低病原性H7N2型はフェレットの上気道で効率的に増殖し、直接接触で感染できることが確認された。	
90064	2008/12/01	80762	鳥インフルエンザ	ProMED-mail20080825.2648	タミフル耐性型の「通常の」季節性インフルエンザが急速に拡大しており、南アフリカでは今年の冬(2008～2009年)のインフルエンザに効果がないおそれがある。WHOのデータによると同国でH1N1株に感染した107名に関する検査の結果、全員がタミフルに耐性の突然変異株を保有していた。2008年4月1日から8月20日に南半球の12カ国のH1N1インフルエンザ感染患者由来検体788例中242例(31%)がタミフル耐性に関係があるH274Y突然変異を有していた。	19
90064	2008/12/01	80762	梅毒	SignOnSanDiego.com 2008年3月26日	カリフォルニア州サンディエゴ郡の年間梅毒症例数は、最低となった2000年の28例から昨年(2007年)は340例まで急増した。州の他の大都市の郡と比べて非常に急激な増加である。増加率は州全体の2倍以上、全国の3倍以上になる。州から派遣された5名の専門家チームは、梅毒と診断された人々と連絡をとって、性的パートナーを探し、検査を受けるよう勧めている。	

別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	赤血球、血小板	研究報告の公表状況	Transfusion (United States) Aug2008, 48 (8) p1602-8	公表国：米国	
販売名(企業名)	－				

研究報告の概要

供血時点にはB型肝炎に関する血清検査で陰性であったが、その後HBV DNAが検出された供血者から血液成分(赤血球、血小板)の輸注を受けた2例の免疫不全患者について報告する。
供血者は39歳男性で、供血時点は血清検査陰性であったが、6週間後に採取した検体では抗HBc抗体陽性(HBs抗原、抗HBs抗体は未検出)となり、その後の検査でHBV DNAが検出された。
1例は化学療法により免疫不全状態にあった重症な急性リンパ性白血病の9歳女児で、HBVワクチンにより低レベルの抗HBs抗体を獲得していたが、赤血球輸注から13ヵ月後に急性B型肝炎を発症した(発症までの間、全てのHBVマーカーは陰性)。感染原因は、供血血液中にHBV DNAが存在し、供血者と受血者の遺伝子配列(pre-S/SとBCP/PC)の同一性から、輸血による感染と確認された。
もう1例は化学療法による免疫不全状態にあった骨髄異形性症候群の65歳女性(HBs抗原、HBc抗体陰性、抗HBs抗体低レベル陽性)で、先の症例と同じ供血者から得た血小板の輸注を受けていたが、感染はしなかった。
両受血者の当該輸血前の低レベル抗HBs抗体の存在は、ウイルス量がHBsAg検出限界であると考えられる1000～3000コピー/mL未満と低いため、保護的役割(感染成立の阻害)を果たした可能性がある。また、両受血者は偶然に別の供血者からの高力価抗HBs抗体を含有する血漿又は血小板を輸注されており、HBVの受動抗体を受けていた。両受血者の違いは、1例目が輸血当日に受動抗体を受けたのに対し、2例目は感染源の血小板を投与されてから3日後に受動抗体を受けたことであった。
比較的少量のHBVに曝露した両受血者では、HBVワクチン接種または受動免疫化による予防、あるいは逆に化学療法や免疫抑制による易感染性などの人的介入により、ウイルス感染の早期段階を古典的に定義する変数がかなり修飾されることが示された。この結果、本報告のように複雑な条件下では、輸血、再燃および院内要素を分けて考える必要があり、その解明には進歩した分子方法が最も有用である。

使用上の注意記載状況・その他参考事項等

重要な基本的注意
(1) 本剤の原材料となる(献血者の)血液については、HBs抗原、抗HCV抗体、・・・・陰性で、かつALT(GPT)値でスクリーニングを実施している。さらに、プールした試験血漿については、HIV-1、HBV及びHCVについて核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該NATの検出限界以下のウイルスが混入している可能性が常に存在する。

報告企業の意見	今後の対応
後にHBV DNA陽性と判明した血液によるB型肝炎感染の報告である。 当社血漿分画製剤の製造工程におけるHBVのモデルウイルスに対するウイルスクリアランス指数は9以上である。なお、原料血漿はミニプール血漿における NAT 検査で HBV DNA 陰性を確認しており、最終製品においても HBV DNA 陰性を確認している。	今後ともにB型肝炎ウイルス感染に関する安全性情報に留意していく。

TRANSFUSION COMPLICATIONS

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

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

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

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

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

Among blood-borne viruses of major concern in transfusion, hepatitis B virus (HBV) presents the highest residual risk,[1] despite several serologic markers available for screening. HBV DNA testing is routinely performed in Germany[2] and Japan[3] and, more recently, in several additional European countries.[4] HBV DNA testing is an expensive alternative to anti-HBc in place for years in several low-prevalence countries but remains cost-prohibitive in areas of higher prevalence to avoid blood shortage. Genomic screening can be performed on individual donations or in plasma pools ranging between 6 and 96, although it was shown that pooling reduces significantly the yield of DNA-containing donations.[4,5] In Brazil, despite relatively high prevalence of the marker, anti-HBc screening is mandatory and a few blood banks also routinely test blood donations for both hepatitis C virus (HCV) and human immunodeficiency virus (HIV) RNA but not for HBV DNA.[6] A fundamental limitation of anti-HBc screening is the inability to detect window-period, highly infectious, donations. The preseroconversion window period has been extensively studied in serial plasma donor samples and typically ranges between 37 and 87 days (median, 59 days).[7] Post-transfusion infection was not systematically investigated but the early stages were assumed to be of similar or shorter duration due to the large volume of the inoculum. The protective effect of anti-HBs has been well established as well as the increased susceptibility to HBV infection of

ABBREVIATIONS: BCP = basic core promoter; PC = precore.

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Received for publication December 18, 2007; revision received February 4, 2008, and accepted February 4, 2008.
doi: 10.1111/j.1537-2995.2008.01723.x
TRANSFUSION 2008;48:1602-1608.

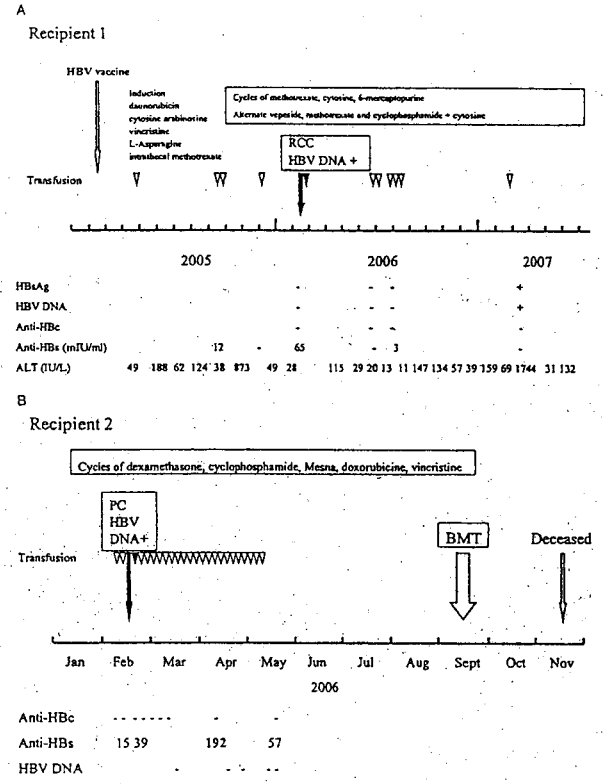

Fig. 1. Case description. (A) Summary of Recipient 1 clinical history. The implicated transfusion of RBCs is indicated by a full arrow. Other transfusions received are indicated as open triangles. The filled triangle indicates the blood product containing high titer of anti-HBs. Bolded ALT levels indicate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

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

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

CASE REPORT

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

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

A plateletpheresis concentrate prepared from the index automatic blood donation of February 23 (Trima, Gambro BCT, Lakewood, CO) was transfused to a second patient (Recipient 2), a 65-year-old female diagnosed with high-risk myelodysplastic syndrome evolving to biphenotypic leukemia. At the time of the suspect transfusion, she was receiving Hyper-CVAD (ondosetin, dexamethasone, cyclophosphamide, Mesna, doxorubicine, and vincristine) plus intrathecal QT (methotrexate and aracytin). She was negative for the presence of HBsAg and anti-HBc but had a low level of anti-HBs (13 mUI/mL). In September 2006, she received marrow transplantation in another hospital where no clinical or laboratory evidence of HBV infection was observed. She died of sepsis in November 2006.

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

TABLE 1. HBV markers in samples from the implicated donor

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

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

MATERIALS AND METHODS

Serologic testing

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

Molecular testing

DNA was extracted from 200 µL of serum and/or plasma with a DNA blood mini kit (QIAamp, Qiagen, Hilden, Germany) in Brazil and either tested locally or shipped to the UK in dry ice. HBV DNA was detected initially by one-step PCR using 7 µL of extract DNA submitted to a fast PCR protocol (Applied Biosystems, Foster City, CA) in the presence of 1 µmol per L of each primer OY1 sense (5'-CAAGGTATGTTGCCCGTTTG-3') and OY2 antisense (5'-AAAGCCCTGACCACTGA-3'),[8] in a final volume of 25 µL. Nested PCR was performed on 12.5 µL of DNA in a 25-µL reaction (final volume) as previously described.[9] All PCR procedures were performed in a thermocycler (Model 9700, Applied Biosystems). Two nested PCR procedures were used to amplify a 276-bp fragment located in the basic core promoter (BCP) and precore (PC) regions and a 1434-bp fragment spanning the whole pre-S/S gene, as previously described.[10] Sequences of BCP/PC and pre-S/S regions were obtained by direct sequencing of amplicons. Sequences were aligned with reference HBV genotype A to H sequences using computer software (Clustal W software implemented in Mac Vector Version 7.2, Accelrys, San Diego, CA), and the alignments were confirmed by visual inspection. Phylogenetic analysis was performed using computer software (PAUP 4.0b10, Sinauer Associates, Inc., Sunderland, MA) after exclusion of positions containing an alignment gap from pairwise sequence comparisons. Nucleotide distances were analyzed by neighbor-joining algorithm based on Kimura two-parameter distance estimation. To confirm the reliability of the phylogenetic trees, bootstrap resampling was performed for each analysis (1000 replicates).

RESULTS

Analysis of the implicated donation sample and donor

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

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

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

Recipient 1

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

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

Recipient 2

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

DISCUSSION

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

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

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

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

While the identical sequence of more than 1500 cumulated bases between donor and recipient HBV strains leaves little doubt about the donor being responsible for the infection, once contamination of the donor sample has been excluded, the discrepancy of the outcome of HBV contact between the two recipients raises multiple questions. Although both patients received chemotherapy accompanied with assumed substantial immunosuppressive effects and similar volumes of HBV DNA–containing plasma (110 and 180 mL for Recipients 1 and 2, respectively), only Recipient 1 developed infection. Neither age nor volume of the inoculum could significantly affect the ability to develop an immune response since, at age 9, the maturity of the immune system is comparable to that of an adult. The presence of low levels of anti-HBs before the implicated transfusion in both recipients might have played a protective role, particularly as the blood component viral load was low, below 1000 to 3000 copies per mL, which is considered the limit of detection for HBsAg.[17,18] Coincidentally, both recipients received passive antibodies to HBV in the form of 125 mL of plasma containing high-titer anti-HBs from the same double-plateletpheresis donation. One difference between the two patients was that Recipient 1 received 125 mL of this plasma the day of transfusion with the implicated product while Recipient 2 received the same volume of plasma 3 days after being in contact with the implicated PLT concentrate. Since the suspected viral strain was wild type in the S region, there is a high likelihood that anti-HBs either raised by vaccine or passively transmitted was neutralizing the circulating virus.

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

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

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

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

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

ACKNOWLEDGMENT

We acknowledge Dr Vicente Odone Filho for referring Recipient 1 for investigation.

REFERENCES

1. Schreiber GB, Busch MP, Kleinman SH, Korelitz JJ. The risk of transfusion-transmitted viral infections. N Engl J Med 1996;334:1685-90.
2. Roth WK, Weber M, Petersen D, Drosden C, Buhr S, Sireis W, Weichert W, Hedges D, Seifried E. NAT for HBV and anti-HBc testing increase blood safety. Transfusion 2002; 42:869-75.
3. Meng Q, Wong C, Rangachari A, Tamatsukuri S, Sasaki M, Fiss E, Cheng L, Ramankutty T, Clarke D, Yawata H, Sakakura Y, Hirose T, Impraim C. Automated multiplex assay system for simultaneous detection of hepatitis B virus DNA, hepatitis C virus RNA, and human immunodeficiency virus type 1 RNA. J Clin Microbiol 2001;39:2937-45.
4. Brojer E, Grabarczyk P, Liszewski G, Mikulska M, Allain J-P, Letowska M. Characterization of HBV DNA positive/HBsAg negative blood donors identified in the Polish NAT screening program. Hepatology 2006;44:1666-74.
5. Owusu-Ofori S, Temple J, Sarkodie F, Anokwa M, Candotti D, Allain JP. Pre-donation screening of blood donors with rapid tests: implementation and efficacy of a novel approach to blood safety in resource-poor settings. Transfusion 2005;45:133-40.
6. Wendel S, Levi JE, Takaoka DT, Silva IC, Castro JP, Torezan-Filho MA, Ghaname J, Gioachini R, Durigon EL. Primary screening of blood donors by NAT testing for HCV-RNA: development of an "in-house" method and results. Rev Inst Med Trop Sao Paulo 2007;49:177-85.
7. Mimms L, Mosley JW, Hollinger FB, Aach RD, Stevens CE, Cunningham M, Vallari DV, Barbosa LH, Nemo GJ. Effect of concurrent acute infection with hepatitis C virus on acute hepatitis B virus infection. BMJ 1993;307:1095-7.
8. Yokosuka O, Tagawa M, Omata M. PCR detection of hepatitis B virus. In: Persing D, editor. Diagnostic molecular microbiology, principles and applications. Washington, DC: ASM Press; 1993. p. 2-3.
9. Allain JP, Candotti D, Soldan K, Sarkodie F, Phelps B, Giacherti C, Shyamala V, Yeboah F, Anokwa M, Owusu-Ofori S, Opare-Sem O. The risk of hepatitis B virus infection by transfusion in Kumasi, Ghana. Blood 2003;101: 2419-25.
10. Candotti D, Opare-Sem O, Rezvan H, Sarkodie F, Allain JP. Molecular and serological characterization of hepatitis B virus in deferred Ghanaian blood donors with and without elevated alanine amino transferase. J Viral Hepat 2006;13: 715-24.
11. Barker LF, Murray R. Relationship of virus dose to incubation time of clinical hepatitis and time of appearance of hepatitis-associated antigen. Am J Med Sci 1972;263: 27-33.
12. Roche B, Samuel D, Gigou M, Feray C, Virot V, Schmets L, David MF, Arulnaden JL, Bismuth A, Reynes M, Bismuth H. De novo and apparent de novo hepatitis B virus infection after liver transplantation. J Hepatol 1997;26:517-26.
13. Prince AM, Lee DH, Brotman B. Infectivity of blood from PCR positive, HBsAg negative, anti-HBs-positive cases of resolved hepatitis B infection. Transfusion 2001;41:329-32.
14. Hyams KC. Risks of chronicity following acute hepatitis B virus infection: a review. Clin Infect Dis 1995;20:992-1000.
15. Motta-Castro AR, Martins RM, Yoshida CF, Teles SA, Panago AM, Lima KM, Gomes SA. Hepatitis B virus infection in isolated Afro-Brazilian communities. J Med Virol 2005;77:188-93.
16. Araujo NM, Mello FC, Yoshida CF, Niel C, Gomes SA. High proportion of subgroup A' (genotype A) among Brazilian isolates of hepatitis B virus. Arch Virol 2004;149:1383-95.
17. Biswas R, Tabor E, Hsia CC, Wright DJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP. Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. Transfusion 2003;43:788-98.
18. Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP. Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. Transfusion 2004;44:1332-9.
19. Yoshikawa A, Gotanda Y, Itabashi M, Mineshigi K, Kanemitsu K, Nishioka K. Hepatitis B NAT virus-positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. Vox Sang 2005;88:77-86.
20. Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Misoguchi H. Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen-negative) infection in the acute stage. Transfusion 2007;47:1162-71.
21. Gerlich WH, Wagner FF, Chudy M, Harrisho] LH, Lattermenn A, Wienzek S, Glebe D, Sanievski M, Schuttler CG, Wend UC, Willems WR, Bauerfeind U, Jork C, Bein G, Platz P, Ullum H, Dickmeiss E. HBsAg non-reactive HBV infection in blood donors: transmission and pathogenicity. J Med Virol 2007;79:S32-S36.
22. Tabor E. Infections by hepatitis B surface antigen gene mutants in Europe and North America. J Med Virol 2006;78:S43-S47. □

別紙様式第2-1

No. 37

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
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	Satoh K, Iwata-Takakura A, Yoshikawa A, Gotanda Y, Tanaka T, Yamaguchi T, Mizoguchi H. Vox Sang. 2008; 95(3): 174-80.	公表国 日本	
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(HBV)DNAおよびHBV表面抗原の新規濃縮方法:オカルトHBV感染検出方法への応用

背景:輸血後B型肝炎ウイルス(HBV)感染のリスクは、HBV核酸増幅技術(NAT)の導入後減少したが、HBV DNA陽性かつ表面抗原(HBsAg)陰性オカルトHBV感染の問題は未解決である。その理由の一つは、オカルトHBV感染はミニプールNATにより検出するにはHBV DNA量が少なすぎることである。HBVコア抗体(HBcAb)の検査は、オカルトHBV感染を完全には排除していない。そのため、検出感度を上げるために、HBV DNAとHBsAgを同時に濃縮する新規方法を開発した。

方法:二価金属存在下でpoly-L-lysineを使用し、ウイルス凝集反応を増強させ、ウイルスを濃縮する。濃縮処理時間を短縮するためにpoly-L-lysineでコートした磁気ビーズ法を用いる。HBcAb陽性およびHBsAg陰性供血血液77本について、酵素免疫法(EIA;AxSYM、Abbott社)および赤血球凝集阻害検査(日本赤十字社)により、HBsAgおよびHBcAbをそれぞれ調べた。

結果:HBV DNAとHBsAg量は、最高4〜7倍に濃縮された。この方法により、HBcAb陽性およびHBsAg陰性供血者77名のうち35名は個別NATにてHBV DNA陽性となり、更に供血者5名はHBVの濃縮によりHBV DNA陽性となった。オカルトHBV感染者40名のうち27名は、HBsAgの濃縮によりHBsAg陽性となった。

結論:HBV DNAおよびHBsAgを濃縮する我々の新しい方法により、EIAとHBV NATの感度が上昇し、HBsAg EIAを用いてオカルトHBV感染者40名のうち27名を検出することができた。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
HBV DNAとHBsAg量を、同時に最高4〜7倍に濃縮することで、EIAとHBV NATの感度が上昇し、HBV DNA量が少ないオカルトHBV感染者40名のうち27名を検出することができたとの報告である。	日本赤十字社では、HBs抗原検査及びHBc抗体検査を実施することに加えて、HBVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法(CLEIA)及び精度を向上させた新NATシステムを導入した。HBV検査に関する新たな知見等について今後も情報の収集に努める。

MedDRA/J Ver.11.0J

(2)

JRC2008T-064

Vox Sanguinis (2008) 95, 174–180
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Journal compilation © 2008 Blackwell Publishing Ltd.
DOI: 10.1111/j.1423-0410.2008.01091.x

ORIGINAL PAPER

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

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Vox Sanguinis

Received: 29 May 2008,
revised 17 July 2008,
accepted 18 July 2008,
published online 5 August 2008

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

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

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

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

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

Introduction

More than 350 million people worldwide are chronically infected with hepatitis B virus (HBV) [1]. HBV is one of the most important viral infections transmitted by transfusion. Nucleic acid amplification technology (NAT) screening has widely been introduced for hepatitis C virus (HCV) and human immunodeficiency virus, and has greatly reduced the risk of transfusion-transmitted infection by these viruses. In contrast, HBV NAT has not been widely implemented, in part due to assay sensitivity issues. HBV therefore remains a source of post-transfusion infection. The risk of post-transfusion HBV infection has been reduced after the implementation of

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HBV NAT in Japan, and other countries reduce the risk of transmission by using assays with increased sensitivity for the detection of HBV surface antigen (HBsAg) [2–8]. These approaches have reduced the window period in the early stage of infection. The problem of occult HBV infection, recently defined as individuals who are HBsAg-negative and HBV NAT-positive regardless of the presence or absence of antibody to hepatitis B core antigen (anti-HBc) and antibody to hepatitis B surface antigen (anti-HBs), however, remains to be solved. Anti-HBc screening of blood donations has reduced the risk of occult HBV infection [9–13]. However, in HBV endemic areas such as Asia, anti-HBc screening is not generally utilized, because the rate of positivity is so high that many blood products would be discarded. One possible solution to this problem is to modify the cut-off value of the anti-HBc test and also to take into account the titre of anti-HBs. Using this approach, the Japanese Red Cross (JRC) has succeeded in reducing the frequency of post-transfusion HBV infections, particularly post-transfusion fulminant HBV infection [14,15]. However, the problem of occult HBV infection has not been completely removed and each year a number of cases of transfusion-associated HBV continue to be reported [16,17]. In an attempt to address this, the cut-off value of anti-HBc has been decreased and the sensitivity of HBV NAT testing increased by reducing the pool size from 50 to 20 and also increasing the input volume for the NAT assay from 0·2 ml to 0·85 ml [15]. However, there are limitations for the strategy from the view point of cost-effectiveness.

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

Materials and methods

Samples

Hepatitis B virus surface antigen-positive and/or anti-HBc-positive donations that did not meet standard JRC requirements were collected with the cooperation of blood centres in the eastern part of Japan from March 2003 to June 2006. None of these donations were used for transfusion purposes. Two hundred and fifty-nine donations were available. These were subdivided into 2·5-ml tubes and stored at –20°C. The remaining plasma from the donation was also stored at –20°C. Of the 259 donations, 182 were HBsAg-positive by enzyme immunoassay (EIA) (AxSYM®; Abbott Laboratories, North Chicago, IL, USA) and 77 were anti-HBc-positive [$\geq 2^5$ by haemagglutination inhibition assay (HI), JRC in-house], HBsAg-negative (EIA; AxSYM®) and anti-HBs-negative [$< 2^4$ (less than 200 mIU/ml)] by passive haemagglutination assay (JRC in-house). An anti-HBc titre $\geq 2^5$ by HI is equal to $\geq 2^7$–2^8-fold diluted sample that is positive (≥ 50% inhibition) by anti-HBc EIA (AxSYM®).

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

Preparation of poly-L-lysine-coated magnetic beads

COOH magnetic beads (125 mg/2·5 ml) (IMMUTEX-MAG™; Japanese Synthetic Rubber, Tokyo, Japan) were added to 0·1 M 2-morphorinoethansulphate (MES) (Wako Pure Chemical, Tokyo, Japan) solution (final volume, 5·0 ml; pH 5·0) and were incubated for 10 min. Activated magnetic beads (25 mg/ml) were suspended in a coupling buffer [5 ml of 100 mM MES (pH 5·0), 50 µl of 100 mg/ml poly-L-lysine (Wako) and 1·2 ml of distilled water] and mixed by continuous inversion at room temperature for 15 min. Then 1·25 ml of 1-ethyl-3-(3-dymethyl-aminopropyl)-carbodiimido (Wako) solution was added to the mixture and mixed by continuous inversion at 10°C for 20 h. Then the solution was replaced with 1 M ethanolamine (Wako) to block reactions at 4°C overnight. Poly-L-lysine-coated magnetic beads were washed five times with phosphate-buffered saline (PBS) and stored at 4°C at a concentration of 50 mg/ml.

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

Concentration of HBsAg and HBV DNA

Poly-L-lysine-coated magnetic beads were added to 2 ml of plasma at a final concentration of 1 mg/ml. Then, 30 µl of 1·1 M $Zn(COOH)_2$ was added to the sample. The resulting mixture was mixed and left to stand for 5 min. The agglutinated HBsAg/HBV DNA and magnetic beads were trapped in a magnetic field (MagicalTrapper®, Toyobo, Tokyo, Japan) and washed twice with PBS to remove impurities. The concentrated HBsAg was eluted with 0·25 ml of 0·4 M ethylenediaminetetraacetic acid (EDTA) solution. The whole volume of the sample was eluted for EIA testing (AxSYM®, Abbott) (effective eightfold concentration). HBV DNA was eluted with 100 µl of 0·4 M EDTA solution and 50 µl or 100 µl was used for individual NAT (10- or 20-fold concentration, respectively). The concentration and elution process takes 30 min.

HBV DNA extraction and quantification

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

the HBV DNA, nucleic acid extracts were amplified and titrated by using a sequence-detection system (TaqMan, ABI Prism 7700 Sequence Detector; PE Applied Biosystems, Foster, CA, USA). Quantification of the HBV DNA was calculated from the working curve (10^7, 10^6, 10^5, 10^4, 10^3 and 10^2 copies/ml) produced by domestic standard samples that were prepared based on the international standard (NIBSC: National Institute for Biological Standards and Control). Calculation was carried out using Sequence Detector version 1·7 (PE Applied Biosystems). The qualitative detection limit was assumed to be 60 copies/ml (95% confidence interval) and quantitative detection limit was assumed to be 100 copies/ml (95% confidence interval).

The AxSYM® HBsAg assay was used for detection of HBsAg. Tests were carried out in accordance with the manufacture's instructions. A positive result is defined as a signal/noise (s/n) ratio ≥ 2. Samples with different concentrations of HBsAg were used to assess the effectiveness of HBsAg concentration. High-titre HBsAg samples (AxSYM®; s/n ratio 266) were sequentially diluted 10-fold up to a final dilution of 10 000-fold using normal plasma. Lower low-titre HBsAg samples (AxSYM®; s/n ratio 12) were diluted up to a final dilution of 1000-fold. Samples known to have HBsAg below the level of detection in the AxSYM assay (s/n ratio 1·7) were diluted to a final dilution of 100-fold. The respective diluted samples were then concentrated eightfold as described above.

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

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

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

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

Results

Hepatitis B virus was concentrated quantitatively by our new method in a broad range of HBV DNA loads. However, the efficacy of concentration varied from sample to sample. The efficacy of concentration (measured value/expected value: original × concentration times) is shown in Table 1. The efficacy of the concentration process decreased from 0·76 to 0·49 as the HBV DNA load increased from 10^3 to 10^6 copies/ml (Table 1).

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

Sample no.	Original (copies/ml)	10-fold concentration (copies/ml)	Efficacy of concentration*
1	1·6 E + 06	7·8 E + 06	0·49
2	4·2 E + 05	2·1 E + 06	0·50
3	9·0 E + 04	5·7 E + 05	0·63
4	2·2 E + 04	1·6 E + 05	0·73
5	4·6 E + 03	3·5 E + 04	0·76

*Efficacy = 10-fold concentration (copies/ml)/original × 10 (copies/ml).

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

Original sample		10-fold concentration		
HBV DNA (copies/ml)	HBsAb (mIU)	HBV DNA (copies/ml)	Efficacy of concentration
120	0	860	0·72
	1024	1400	1·17
	1792	1300	1·08

The efficacy of HBsAg concentration is shown in Fig. 1. For the high-titre HBsAg samples (s/n ratio 266·03), 100-fold dilution samples were more than limit for detection (s/n ratio 4·88) and 1000-fold dilution samples were less than the limit for detection (s/n ratio 1·16). Following eightfold concentration of HBsAg, the 1000-fold dilution sample was found positive (s/n ratio 3·24). Similarly, in the low-titre sample the undiluted sample was above the detection limit (s/n ratio 11·91). The 10 times dilution sample (s/n ratio 1·69) was negative but became positive following eightfold concentration (s/ratio 4·36). The negative samples (s/n ratio 1·66) became positive by eightfold concentration (s/n ratio 3·49). Based on the parallel translation of linear line shown in Fig. 1, the relative efficacy of concentration was about 0·64(5·1/8) in high-titre samples and 0·56(4·5/8) in low-titre samples.

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

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

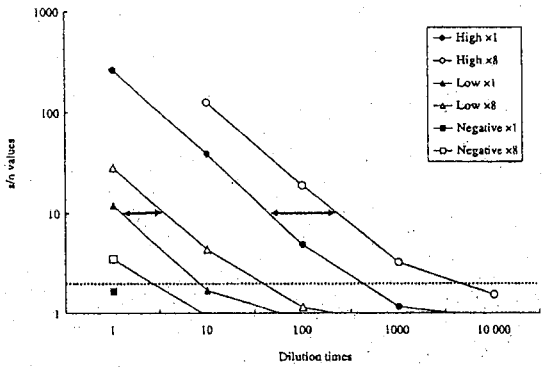

Fig. 1 Parallel translation of linear line caused by hepatitis B surface antigen (HBsAg) concentration. Vertical axis shows signal/noise (s/n) values of enzyme immunoassay (EIA) indicated by logarithm, and horizontal axis shows dilution fold of samples indicated by logarithm. The linearity was observed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

Data for Fig.1

		HBsAg: EIA(AxSYM: s/n^{-1}) dilution with normal plasma				
		1	10	100	1000	10 000
High	×1	266·03	38·81	4·88	1·16	0·91
	×8		126·77	18·95	3·24	1·54
Low	×1	11·91	1·69	0·86	0·77	
	×8	28·28	4·36	1·15	0·76	
Negative	×1	1·66				
	×8	3·49	0·93	0·8		

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

Plasma for dilution	AxSYM (s/n[b]) HBsAg dilution with various kinds of plasma[a]	10-fold concentration of diluted HBsAg plasma
Normal plasma	1·39	3·80
HCV-positive plasma[c]	1·18	3·47
Parvovirus B19-positive plasma[d]	1·31	3·77

[a]The original HBsAg-positive plasma titre is 6·19: EIA (AxSYM; s/n).
[b]More than 2 means positive.
[c]The titre of anti-HCV was > 2^{12} and the load of HCV RNA was 10^6 copies/ml.
[d]The titre of B19 antigen was 2^{11} by receptor-mediated-haemagglutination assay.

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

Seventy-seven anti-HBc positive (≥ 2^5 by HI assay by JRC criteria) and HBsAg-negative (EIA, AxSYM®) donations were selected to study the efficacy of HBsAg and HBV DNA concentrations. Of the 77 samples, 35 were positive by individual NAT and a further five became NAT positive following concentration (Table 4). Of 35 samples (Table 4; lanes d, e), 16 (Table 4; lane e) had HBV DNA loads of 120–1500 copies/ml and the other 19 samples (Table 4; lane d) had HBV DNA loads less than the quantitative detection limit (< 100 copies/ml). However, the HBV DNA loads of all these samples exceeded 100 copies/ml following concentration (Table 4; lanes d, e). Five samples (Table 4; lanes b, c) that were negative by individual NAT became positive (less than 100–510 copies/ml) following concentration.

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

				HBV DNA (copies/ml)				
				a	b	c	d	e
			Original	Negative	Negative	Negative	< 100	≥ 100
			Concentration (×20)	Negative	< 100	≥ 100	≥ 100	NT
HBsAg (AxSYM)	I	Original	Negative	34	0	0	8	5
		Concentration (×8)	Negative					
	II	Original	Negative	3	1	4	11	11
		Concentration (×8)	Positive					

NT, not tested.

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

Discussion

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

It has previously been reported that NAT sensitivity can be increased by reducing the number of donations in the mini-pool [23], increasing the input volume of serum, and by addition of an ultracentrifugation step [24]. From the viewpoint of cost-effectiveness, an inexpensive and easy method to increase sensitivity is desirable. We have previously reported a virus concentration method using polyethyleneimine [25]. However, HBV DNA and HBsAg were not concentrated qualitatively by the method, because the combination of extracted nucleic acids of viruses and magnetic beads is difficult to dissociate in the presence of protein-degenerative reagents. We have solved this problem with the use of poly-L-lysine that coagulates with viruses in the presence of bivalent metal ions (zinc acetate).

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

It is important to determine whether HBV could be concentrated in the presence of anti-HBs. In this study, HBV was much more efficiently concentrated in the presence of anti-HBs than without (Table 2). The results showing that the efficacy of concentration was more than 1·0 might be a result of the easy coagulation of antigen antibody-reacted materials with poly-L-lysine beads. However, in the case of HBsAg concentration, it is difficult to measure the efficacy of HBsAg concentration in the presence of anti-HBs, because anti-HBs inhibits the detection of HBsAg by EIA. The coexistence of other viruses would not affect the concentration of HBsAg/HBV DNA, as shown in Table 3. Moreover, the procedure is useful for concentrating coinfected enveloped viruses as HCV, although it will be difficult to concentrate non-enveloped viruses as parvovirus B19. HCV that is difficult to concentrate by ultracentrifugation because of its low density is easily concentrated quantitatively by our method.

We succeeded in concentrating HBsAg from occult HBV infection. The theoretical plasma HBsAg concentration was eightfold (2 ml of plasma/0·25 ml of elution); however, from the parallel translation of the linear line (vertical axis - s/n

and horizontal axis – dilution folds of samples), the relative efficacies of concentration were 0·56–0·64. The reason for the low efficacy of HBsAg concentration compared to the efficacy of HBV DNA concentration (0·49–0·76) might be due to HBsAg (22 nm) being smaller than HBV (45 nm) and thus the efficacy of agglutination with poly-L-lysine being different.

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

Although HBsAg-negative subjects may retain a low infectivity and have a low risk for progressive liver damage [17], HBV DNA testing or an HBsAg detection method with the highest sensitivity should be implemented to decrease the risk of post-transfusion HBV infection [26,27]. Our new HBV/HBsAg concentration method could contribute to increasing the sensitivity of HBV DNA/HBsAg detection. The concentration method could be combined with either Chemoluminescent Immunoassay (CLIA; PRISM, Abbott) or individual donation NAT to further increase the overall sensitivity of HBV detection. Alternatively, if a high-sensitivity method such as the CLIA was combined with our method, then it might be possible to undertake screening using pooled samples. Our concentration method would potentially be capable of replacing individual NAT by mini-pool NAT, although the present efficacy of concentration is not 1·0 but about 0·7 (Table 1).

Acknowledgements

We gratefully acknowledge the guidance and support of the President of JRC, Mr T Konoe, the Executive Counsellor of JRC, Dr K Tadokoro, and Emeritus Professor Dr M Mayumi of Jichi Medical School. We wish to thank Dr Peter Flanagan for language supervision.

References

1 Lavanchy D: Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; 11:97–107

2 Yoshikawa A, Gotanda Y, Itabashi M, Minegishi K, Kanemitsu K, Nishioka K; Japanese Red Cross NAT Screening Research Group: HBV NAT positive blood donors in the early and late stages of HBV infection: analyses of the window period and kinetics of HBV DNA. *Vox Sang* 2005; 88:77–86

3 Minegishi K, Yoshikawa A, Kishimoto S, Yugi H, Yokoya N, Sakurada M, Kiyokawa H, Nishioka K; Japanese Red Cross NAT Research Group: Superiority of minipool nucleic acid amplification technology for hepatitis B virus over chemiluminescence immunoassay for hepatitis B surface antigen screening. *Vox Sang* 2003; 84:287–291

4 Mine H, Emura H, Miyamoto M, Tomono T, Minegishi K, Murokawa H, Yamanaka R, Yoshikawa A, Nishioka K; and Japanese Red Cross NAT Screening Research Group: High throughput screening of 16 million serologically negative blood donors for hepatitis B virus, hepatitis C virus and human immunodeficiency virus type-1 by nucleic acid amplification testing with specific and sensitive multiplex reagent in Japan. *J Virol Methods* 2003; 112:145–151

5 Biswas R, Tabor E, Hsia CC, Wright JJ, Laycock ME, Fiebig EW, Peddada L, Smith R, Schreiber GB, Epstein JS, Nemo GJ, Busch MP: Comparative sensitivity of HBV NATs and HBsAg assays for detection of acute HBV infection. *Transfusion* 2003; 43:788–798

6 Lelie N, Heaton A: Hepatitis B – a review of the role of NAT in enhancing blood safety. *J Clin Virol* 2006; 36:S1–S2

7 Kleinman SH, Busch MP: Assessing the impact of HBV NAT on window period reduction and residual risk. *J Clin Virol* 2006; 36:S23–S29

8 Kleinman SH, Strong DM, Tegtmeier GG, Holland PV, Gorlin JB, Cousins CR, Chiacchierini RP, Pietrelli LA: Hepatitis B virus (HBV) DNA screening of blood donations in minipools with the COBAS AmpliScreen HBV test. *Transfusion* 2005; 45:1247–1257

9 Kleinman SH, Kuhns MC, Todd DS, Glynn SA, McNamara A, DiMarco A, Busch MP: Frequency of HBV DNA detection in US blood donors testing positive for the presence of anti-HBc: implications for transfusion transmission and donor screening. *Transfusion* 2003; 43:696–704

10 Roth WK, Weber M, Petersen D, Drosten C, Buhr S, Sireis W, Weichert W, Hedges D, Seifried E: NAT for HBV and anti-HBc testing increase blood safety. *Transfusion* 2002; 42:869–875

11 Allain JP: Occult hepatitis B virus infection: implications in transfusion. *Vox Sang* 2004; 86:83–91

12 Panhotra BR, Bahrani A, Joshi CS, Hassan Z: Occult hepatitis B virus infection among anti-HBc positive blood donors: Necessitates substitution of screening by HBV NAT. *J Infect* 2005; 51:263

13 Hennig H, Puchta I, Luhm J, Schlenke P, Goerg S, Kirchner H: Frequency and load of hepatitis B virus DNA in first-time blood donors with antibodies to hepatitis B core antigen. *Blood* 2002; 100:2637–2641

14 Iizuka H, Ohmura K, Ishijima A, Satoh K, Tanaka T, Tsuda F, Okamoto H, Miyakawa Y, Mayumi M: Correlation between anti-HBc titers and HBV DNA in blood units without detectable HBsAg. *Vox Sang* 1992; 63:107–111

15 Yugi H, Mizui M, Tanaka J, Yoshizawa H: Hepatitis B virus (HBV) screening strategy to ensure the safety of blood for transfusion through a combination of immunological testing and nucleic acid amplification testing-Japanese experience. *J Clin Virol* 2006; 36:S56–S64

16 Inaba S, Ito A, Miyata Y, Ishii H, Kajimoto S, Tanaka M, Maruta A, Saito S, Yugi H, Hino M, Tadokoro K: Individual nucleic amplification technology does not prevent all hepatitis B virus transmission by blood transfusion. *Transfusion* 2006; 46:2028–2029

17 Satake M, Taira R, Yugi H, Hino S, Kanemitsu K, Ikeda H, Tadokoro K: Infectivity of blood components with low hepatitis B virus DNA levels identified in a lookback program. *Transfusion* 2007; 47:1197–1205

18 Yoshikawa A, Gotanda Y, Minegishi K, Taira R, Hino S, Tadokoro K, Ohnuma H, Miyakawa K, Tachibana K, Mizoguchi H; Japanese Red Cross NAT Screening Research Group: Lengths of hepatitis B viremia and antigenemia in blood donors: preliminary evidence of occult (hepatitis B surface antigen-negative) infection in the acute stage. *Transfusion* 2007; 47:1162–1171

19 Kuhns MC, Kleinman SH, McNamara AL, Rawal B, Glynn S, Busch MP: Lack of correlation between HBsAg and HBV DNA levels in blood donors who test positive for HBsAg and anti-HBc: implications for future HBV screening policy. *Transfusion* 2004; 44:1332–1339

20 Allain JP, Sarkodie F, Candotti D, Opare-Sem O: Lack of correlation between hepatitis B surface antigen and hepatitis B virus DNA levels in blood donors. *Transfusion* 2005; 45:1039–1040

21 Stramer SL: Pooled hepatitis B virus DNA testing by nucleic acid amplification: implementation or not. *Transfusion* 2005; 45:1242–1246

22 Weusten JJAM, van Drimmelen HAJ, Lelie PN: Mathematic modeling of the risk of HBV, HCV, and HIV transmission by window-phase donations not detected by NAT. *Transfusion* 2002; 42:537–548

23 Bouchardeau F, Girault A, Razer A, Servant-Delmas A, Mercier M, Laperche S: Sensitivity of hepatitis B virus DNA transcription-mediated amplification testing in hepatitis B surface antigen-positive blood donations. *Transfusion* 2006; 46:2047–2052

24 Bartolomé J, López-Alcorocho JM, Castillo I, Rodriguez-Iñigo E, Quiroga JA, Palacios R, Carreño V: Ultracentrifugation of serum samples allows detection of hepatitis C virus RNA in patients with occult hepatitis C. *J Virol* 2007; 81:7710–7715

25 Satoh K, Iwata A, Murata M, Hikata M, Hayakawa T, Yamaguchi T: Virus concentration using polyethyleneimine-conjugated magnetic beads for improving the sensitivity of nucleic acid amplification tests. *J Virol Methods* 2003; 114:11–19

26 Wang JT, Lee CZ, Chen PJ, Wang TH, Chen DS: Transfusion-transmitted HBV infection in an endemic area: the necessity of more sensitive screening for HBV carriers. *Transfusion* 2002; 42:1592–1597

27 Dufour DR: Hepatitis B surface antigen (HBsAg) assays – are they good enough for their current uses? *Clin Chem* 2006; 52:1457–1459

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
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	石田 高司、坂野 章吾、森 芙美子、伊藤 旭、李 政樹、稲垣 淳、楠本 茂、小松 弘和、神谷 忠、柚木 久雄、田中 靖人、溝上 雅史、飯田 真介、上田 龍三. 第70回 日本血液学会総会; 2008 Oct 10-12; 京都市.	公表国: 日本	
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	使用上の注意記載状況・その他参考事項等
○20プールNAT導入後、初めて確認された輸血によるHCV感染の一例 症例は新規に最重症再生不良性貧血と診断された54歳の女性で、2007年6月20日に初回輸血が実施され、初回輸血前感染症検査はHCV抗体陰性、HCVコア蛋白陰性であった。10月1日の輸血後感染症検査でHCVコア蛋白の陽性化[28,183.1 fmol/L（<20.0）]が明らかとなったため、血液センターに連絡し遡及調査を開始した。初回輸血前感染症検査残余の保存血清でHCV-RNAが陰性であることを確認した（PCR）。患者には6月20日から10月1日の間に合計54本の赤血球濃厚液または濃厚血小板輸血があり、保管54検体についてHCV個別NAT（核酸増幅法）を施行したところ、2007年8月17日輸血の赤血球濃厚液からHCV-RNAを検出した。患者と献血者のHCV Core-E1-E2領域（1,279bp）の塩基配列をdirect sequence法で決定し、比較した結果両者は一致した。この結果、本症例は輸血によるHCV感染である可能性が極めて高いと結論した。 日本では1999年7月から献血血液の感染症検査に500プールNATを導入し、2000年には50プール、2004年には20プールとしてきた。世界で最も先進的かつ高感度システムといえる。20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本報告が初であり、NAT陰性献血血液からでもHCV感染が成立しうることが示された。 また、患者はHCV混入血の輸血から肺炎で死亡されるまでの約7ヵ月間、HCV抗体価が陽性になることはなく、10月24日以降HCVコア蛋白値は一貫して施設測定可能上限50,000.0以上であった。免疫抑制状態の患者に対するHCV感染については、輸血前後のスクリーニング検査としてHCVコア蛋白が必要である。	人全血液-LR「日赤」 照射人全血液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク 自発報告： 2007年10月19日付1-07000104

報告企業の意見	今後の対応
日本において、プールNAT導入後3例の輸血によるHCV感染症例があるが、本症例は20プールNAT導入後初めて確認された輸血によるHCV感染の報告である。	日本赤十字社では、HCV抗体検査を実施することに加えて、HCVについて20プールでスクリーニングNATを行い、陽性血液を排除している。また、これまでの凝集法と比べて、より感度の高い化学発光酵素免疫測定法（CLEIA）及び精度を向上させた新NATシステムを導入した。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。

③

MedDRA/J Ver.11.0J

OS-1-40 血液疾患患者における末梢血細菌・真菌PCR検査の有用性の検討
PCR analysis of blood for diagnosis of bacterial and fungal infection in hematological patients

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【目的】血液疾患の感染症治療における末梢血の細菌・真菌PCR検査の有効性につき前向きに検討した。【方法】2007年4月より当院で化学療法あるいは造血幹細胞移植を受けた白血病患者のうち、同意が得られた延べ8人に対して、定期的（1週間毎）にまたは発熱時に末梢血の細菌・真菌PCR検査と血液培養を施行した。PCR結果は原則的に非開示とした。【結果】全例経過中に発熱がみられた。PCR検査は延べ14回陽性（細菌13回、真菌1回）、血液培養は延べ6回陽性で（すべて細菌）、そのうち3回で両方陽性となった。なお、連続陽性は1回とカウントした。培養でのみ陽性となった3回すべてで検出されたのは皮膚常在菌であり臨床的にもcontaminationと考えられた。培養とPCRの両方で細菌が検出された3回のうち、1回は同時期の血液で、2回は培養陽性となる2、9日前の血液ですでにPCR陽性であった。細菌PCRのみ陽性であった10回のうち8回は臨床経過から感染の原因菌と考えられたが、経験的抗生剤治療により多くは解熱が得られていた。しかし、Stenotrophomonas maltophiliaが同定された1回では全身状態が増悪したため結果を開示し、抗生剤の変更により改善がみられた。真菌PCRのみ陽性の1回では、臨床的に侵襲性肺アスペルギルス症と診断される20日前からAspergillus fumigatusが検出されていた。【結論】細菌感染の多くは、血液培養の結果あるいは経験的抗生剤投与により治療可能であった。しかし、血液培養が陽性となる前からPCR陽性となっていたケースや、血液培養では検出されずPCRでのみ陽性のケースもみられ、細菌PCRの結果を参考に、より早期から確実に原因菌を想定した抗生剤治療が開始できていた可能性がある。また、真菌感染症においても、血液PCRの結果が臨床経過の改善に有用な症例があることが示唆された。今後さらに多くの症例で、細菌・真菌PCR検査の臨床的有効性を前向きに検討することが必要であると考えられた。

OS-1-41 LevofloxacinとPolymyxin Bを消化管殺菌として好中球減少期に投与された血液悪性疾患119例での感染症合併
Infections in neutropenic patients who received prophylactic Levofloxacin or Polymyxin B

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【背景】Giampaoloらは血液悪性疾患を含む担癌患者への化学療法において、プラセボと比較してLevofloxacin（LVFX）が細菌感染予防に有用である、と報告した。（NEJM, 2006）このような報告を受け、血液悪性疾患治療における好中球減少期の消化管殺菌として、非吸収性のPolymyxin B（PMB）に代わり、LVFXが用いられることが多くなったが、この二剤の感染予防効果の差については不明な点が多い。当科では消化管殺菌として、1999年4月から2005年6月まではPMBを、その後現在まではLVFXを使用してきた。この二剤投与下での感染症などについて比較検討した。【患者と方法】対象は当科で血液悪性疾患に対する治療を受けた119例で、PMB群66例、LVFX群53例。年齢・性別に差はなく、疾患はPMB群がNHL46例、MM13例、HL3例、その他4例、LVFX群がAML15例、ALL12例、NHL12例、MDS5例、MM3例、その他6例。治療はPMB群が自家移植64例、同種移植2例、LVFX群が化学療法21例、自家移植11例、同種移植21例。移植前疾患状態はPMB群がCRまたはPR 61例、その他5例、LVFX群がCRまたはPR 35例、その他18例。好中球減少期に38度以上の発熱が生じた際には各種培養を行うと共に、発熱性好中球減少症のガイドラインに基づいて点滴抗生剤や抗真菌剤の投与を行った。【結果】好中球1000/μl以下の期間はPMB群11 ± 4日、LVFX群18 ± 12日と有意にLVFX群で長かった。血液培養陽性はPMB群7例（グラム陽性菌3例、陰性菌4例）、LVFX群5例（グラム陽性菌4例、陰性菌1例）。感染によりPMB群でのみ2例が死亡した。38度以上の発熱期間、点滴抗生剤の使用、最大CRP値などには二群間で差を認めなかった。【考察】今回の検討は患者背景も異なり、直接の比較ではないが、LVFX群で感染に不利と思われる因子が多いにも関わらず、検討したパラメータでは少なくとも同等ないしは勝っており、LVFXの血液悪性疾患における消化管殺菌としての有用性が示唆された。

OS-1-42 20プールNAT導入後、初めて確認された輸血によるHCV感染の一例
The first case of transfusion-transmitted HCV infection slipping through the 20-member-pool NAT

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症例は新規に最重症再生不良性貧血と診断された54歳女性。初回輸血前感染症検査でHCV抗体陰性、HCVコア蛋白陰性。6月20日初回輸血。2007年10月1日の輸血後感染症検査でHCVコア蛋白の陽性化［28183.1 fmol/L（<20.0）］が明らかとなった。直ちに血液センターに報告し遡及調査を開始。はじめに患者の初回輸血前感染症検査残余の保存血清でHCV-RNAが陰性であることを確認した（PCR）。初回輸血から10月1日の間に合計54本のRCCまたはPC輸血があった。それら対象の保管54検体についてそれぞれHCV個別NAT（核酸増幅法）を施行、うち1検体（2007年8月17日輸血RCC）からHCV-RNAを検出した。患者HCVと献血者のHCV Core-E1-E2領域（1279bp）の塩基配列をdirect sequence法で決定し、比較した結果両者は一致した。この結果、本症例は輸血によるHCV感染である可能性が極めて高いと結論した。日本では1999年7月から献血血液の感染症検査に500プールNATを導入し、2000年には50プールに、2004年からは20プールNATとし、そのスクリーニング感度を上げてきた。世界で最も先進的かつ高感度システムといえる。20プールNAT陰性献血血液由来の血液製剤からのHCV感染の報告は本報告が初である。本発表の第1のメッセージは【NAT陰性献血血液由来の血液製剤からでもHCV感染が成立しうる】ことである。また、本症例は2007年10月17日に同種骨髄移植を施行し、2008年3月30日に肺炎のため死亡された。HCV混入血の輸血から約7ヶ月の全経過でHCV抗体価が陽性になることはなく、10月24日からはHCVコア蛋白値は一貫して施設測定可能上限50000.0以上であった。すなわち、免疫抑制状態の患者に対するHCV感染についてはHCV抗体検査のみでは不十分であることを意味する。これらの事実から、第2のメッセージは【輸血前後のスクリーニング検査としてHCVコア蛋白が必要である】ことである。本症例をふまえ、発表当日は【血液製剤の安全性】について議論したい。

別紙様式第2-1

No. 37

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
				2008. 9. 18	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	Aaron S, McMahon JM, Milano D, Torres L, Clatts M, Tortu S, Mildvan D, Simm M. Clin Infect Dis. 2008 Oct 1;47(7):931-4.	公表国 米国	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要	使用上の注意記載状況・その他参考事項等
○C型肝炎ウイルスの鼻腔内伝播:ウイルス学的および臨床的エビデンス 汚染した薬物吸引器具によるC型肝炎ウイルス(HCV)の鼻腔内伝播の可能性が考えられてはいるが、ウイルス感染源として確定されていない。ニューヨーク市のコミュニティ・クリニックから18歳以上で血液中のHCV PCR陽性の吸引用麻薬常用者38名をリクルーティングした。鼻汁検体を採取したほか、被験者が通常薬物を使用する時のようにストローを使用し、このストローを回収して、血液及びHCV RNAの存在を調べた。鼻汁検体28(74%)、ストロー3(8%)で血液が検出された。HCV RNAは鼻汁検体5(13%)、ストロー2(5%)で検出された。被験者のうち11名では、鼻中隔穿孔など慢性的薬物吸引と関連する鼻の異常が見られた。鼻汁検体と薬物吸引器具に血液とHCV RNAが存在することから、HCV鼻腔内伝播のウイルス学的妥当性が示された。	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
汚染した薬物吸引器具によるC型肝炎ウイルスの鼻腔内伝播のウイルス学的妥当性を示したとの報告である。	HCV感染の新たな伝播ルート等について、今後も情報の収集に努める。

(4)

JRC2008T-055

Intranasal Transmission of Hepatitis C Virus: Virological and Clinical Evidence

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

Hepatitis C virus (HCV) is the most common bloodborne pathogen in the United States and is a major cause of liver-related morbidity, mortality, and liver transplantation [1]. HCV is transmitted through contact with infected blood [2] (mostly via shared needles and other drug injection paraphernalia); however, a large proportion (up to 20%) of HCV infections remain unexplained, especially among noninjection drug users [3]. One hypothesis to account for these unexplained cases involves intranasal transmission of HCV via contaminated implements, such as straws, used to snort cocaine, heroin, and other powdered drugs [4]. Implements inserted into the nasal cavity, which has been eroded by long-term drug sniffing, might come into contact with HCV-infected mucus or blood, which might then be transmited to a susceptible individual sharing the same implement [5]. Epidemiological studies of intranasal transmission of HCV have produced inconsistent findings [6, 7], in part because of the high correlation between drug sniffing and other risk factors for HCV infection. Here, we attempt to refute the intranasal transmission hypothesis by invalidating ≥1 of its virological preconditions. Specifically, we address 2 primary research questions: (1) Does HCV RNA exist in the nasal secretions of serum-positive drug sniffers? (2) If so, can HCV RNA be transferred onto the sniffing implements shared by intranasal drug users. A secondary aim was to examine clinical nasal pathologies that might facilitate intranasal HCV transmission.

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

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

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

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

Received 26 March 2008; accepted 29 May 2008; electronically published 2 September 2008.

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Clinical Infectious Diseases 2008; 47:931–4

1058-4838/2008/4707-0013$15.00
DOI: 10.1086/591693

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

Assay	No. (%) of persons (n = 38)	95% CI
Blood detection with OBTI		
Nasal secretions	28 (73.7)	57.6–85.2
Sniffing straws	3 (7.9)	2.0–21.5
HCV RNA detection with PCR		
Nasal secretions	5 (13.2)	5.3–27.8
Sniffing straws	2 (5.3)	0.5–18.2

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

HCV cDNA was amplified from randomly selected HCV-positive blood samples with use of high-fidelity Pfu polymerase (Perkin Elmer) using 410R and 406F primers and cloned into a TA cloning vector (Invitrogen). The pTA_HCV was used to prepare standard curves ranging from 1×10^4 to 10 copies of HCV mRNA, which were run in parallel to each set of samples. The intensity of DNA bands was evaluated by densitometry using the Kodak Image Analysis System; the HCV load for the test sample was calculated on the basis of the numeric value derived from the HCV titration curve. HCV load was calculated as the number of copies per milliliter for blood specimens and as the number of copies per sample for nasal secretions and implements.

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

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

Sample prevalences of HCV RNA and occult blood in nasal secretions and on sniffing implements were estimated. Ninety-five percent CIs were calculated around point estimates using the adjusted Wald method. Descriptive statistics were calculated for sample descriptors and measures of nasal pathology. Our limited sample size precluded statistical tests of significance (e.g., associations between virological and clinical variables).

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

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

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

Discussion. Our findings revealed a high prevalence of blood (74%) in the nasal secretions of HCV-positive long-term drug sniffers. We also confirmed that HCV RNA was present in the nasal secretions of a substantial proportion (13%) of this cohort. Most significantly, this study demonstrated that both blood and HCV particles can be transferred onto sniffing implements (i.e., straws) during simulated intranasal drug use. Studies have shown that HCV can remain viable on environmental surfaces for up to 16 h, but little is know about the quantity of virus required for transmission [12]. The prevalences of HCV in the nasal secretions and on sniffing straws are likely conservative estimates. It is reasonable to assume that HCV will be present in the nasal secretions with greater frequency and quantity during episodes of active drug sniffing, which may exacerbate discharge of nasal fluids and blood.

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

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

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

Symptom	No. (%) of subjects (n = 38)
Findings of an anterior nasal clinical examination	
Loss of nasal hairs	4 (10.5)
Rhinitis	27 (71.1)
Rhinosinusitis	4 (10.5)
Presence of nasal crusting and/or scabbing	6 (15.8)
Sores or erosion of nasal mucosa	3 (7.9)
Saddlenose deformation	6 (15.8)
Nasopalatal perforation	1 (2.6)
Nasal septum perforation	4 (10.5)
Self-reported nasal pathology	
Frequency of nosebleeds in the past year	
Never, or rarely	26 (68.4)
Once or a few times per month	9 (23.7)
Once or a few times per week	2 (5.3)
Once or more per day	1 (2.6)
Experienced a runny or stuffy nose in the past year	
Never or rarely	16 (42.1)
Once or a few times per month	6 (15.8)
Once or a few times per week	13 (34.2)
Once or more per day	3 (7.9)
Reason for nasal symptoms	
Allergies	19 (60.0)
Cold or influenza	10 (26.3)
Drug sniffing	21 (55.3)
"Have you ever noticed any of the following problems with your nose due to drug sniffing?"	
Scabs in the nose	14 (36.8)
Sores in the nose	8 (21.1)
Poor sense of smell	13 (34.2)
Sinus pain	13 (34.2)
Headaches located in the forehead	16 (42.1)
Double vision	5 (13.2)
"Has a doctor or other health care professional ever told you that the inside of your nose is damaged in any way from sniffing drugs?"	7 (18.4)

Data in table 1 contradict the assumption that, in persons with HCV-positive serum specimens, detection of blood implies the presence of HCV. This discrepancy may be explained by 2 factors. First, the 2 assays (PCR and OBTI) were not performed on the same samples. Second, the OBTI assay for blood detects immune complexes between human hemoglobin (hHb) and monoclonal anti-hHb antibodies, which can occur even in the absence of viable cells. In contrast, PCR can only detect HCV RNA from intact particles. Therefore, the discrepancy between the high prevalence of occult blood and relatively low detection of HCV RNA in nasal secretions may be associated with the rapid deterioration of viral RNA in the nasal environment or the destruction of viral particles by mucosal immunity. If the viability of HCV particles in nasal secretions is moderated by nasal pathology or immunity, this might help explain conflicting epidemiological findings in which these moderating factors are not considered.

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

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

Acknowledgments

We thank Enrique Pouget for assistance with data management and Jeanine Botta for providing clerical and technical assistance. Dr. K. K. Lam provided guidance on manuscript revision.

Financial support. The National Institutes of Health, National Institute on Drug Abuse, and the National Institute of Diabetes and Digestive and Kidney Diseases (R21 DA019834 to J.M.M.).

Potential conflicts of interest. All authors: no conflicts.

References

1. Hollinger FB. Factors contributing to the evolution and outcome of cirrhosis in hepatitis C. Clin Liver Dis 1999; 3:741–55.
2. Memon MI, Memon MA. Hepatitis C: an epidemiological review. J Viral Hepat 2002; 9:84–100.
3. Tortu S, Neaigus A, McMahon JM, Hagen D. Hepatitis C among non-injecting drug users: a report. Subst Use Misuse 2001; 36:523–34.
4. Conry-Cantilena C, van Raden M, Gibble J, et al. Routes of infection, viremia, and liver disease in blood donors found to have hepatitis C virus infection. N Engl J Med 1996; 334:1691–6.
5. McMahon JM, Simm M, Milano D, Clatts M. Detection of hepatitis C virus in the nasal secretions of an intranasal drug-user. Ann Clin Microbiol Antimicrob 2004; 3:1–4.
6. McMahon JM, Tortu S. A potential hidden source of hepatitis C infection among noninjecting drug users. J Psychoactive Drugs 2003; 35: 455–60.
7. Scheinmann R, Hagan H, Lelutiu-Weinberger C, et al. Non-injection drug use and Hepatitis C virus: a systematic review. Drug Alcohol Depend 2007; 89:1–12.
8. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 1987; 162:156–9.
9. Dykewicz M. Clinical approach to diagnosis and treatment of nonallergic rhinitis. Clin Allergy Immunol 2007; 19:335–50.
10. Rosenfeld RM, Andes D, Bhattacharyya N, et al. Clinical practice guideline: adult sinusitis. Otolaryngol Head Neck Surg 2007; 137(Suppl 3): S1–31.
11. Villa PD. Midfacial complications of prolonged cocaine snorting. J Can Dent Assoc 1999; 65:218–23.
12. Krawczynski K, Alter MJ, Robertson BH, Lu L, Spelbring JE, McCaustland KA. Environmental stability of hepatitis C virus (HCV): viability of dried/stored HCV in chimpanzee infectivity studies. Hepatol 2003; 38(Suppl 4):428.

別紙様式第２－１

医薬品　研究報告　調査報告書

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

(5)

Vox Sanguinis (2008) 95, 94–100

DOI: 10.1111/j.1423-0410.2008.01078.x

ORIGINAL PAPER

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

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Vox Sanguinis

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

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

Results Hepatitis E virus isolates in albumin solutions were inactivated slowly at 60°C for 5 h and the resultant log reduction factor (LRF) was from 1·0 to ≥ 2·2, whereas the virus in PBS was inactivated quickly to below the detection limit and the LRF was ≥ 2·4 to ≥ 3·7. The virus in a freeze dried fibrinogen containing trisodium citrate dihydrate and L-arginine hydrochloride as stabilizers was inactivated slowly and the LRF was 2·0 and 3·0, respectively, of the 72 h at 60°C, but inactivated to below the detection limit within 24 h at 80°C with an LRF of ≥ 4·0. The virus in PBS was also confirmed as to be approximately 35 nm in diameter by nanofiltration. These results are useful for evaluating viral safety against HEV contamination in blood products.

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

Received: 7 February 2008,
revised 26 May 2008,
accepted 1 June 2008

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

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Introduction

Hepatitis E virus (HEV), classified in the genus *Hepevirus*, is a causative agent of human hepatitis. The virus capsid is non-enveloped and the nucleocapsid containing positive-sense single-stranded RNA has a diameter of 27–34 nm [1]. HEV is also endemic in humans, swine and several wild animals such as deers and boars, suggesting that hepatitis E is a zoonosis [2,3].

The virus has been shown to be transmitted by faecal-oral, food-borne and blood-borne routes [1,4–7]. Four genotypes of HEV that infect humans have been identified, three of which, genotypes 1, 3 and 4, have also been isolated from swine and commercial swine liver [1,8,9]. Zoonotic food-borne transmission of HEV was shown to be one reason for the occurrence of a severe form of hepatitis E in Hokkaido, Japan, and HEV genotype and the presence of an underlying disease influenced the severity of the hepatitis E infection [10]. In addition, the prevalence of HEV RNA or anti HEV immunoglobulin G (IgG)-positive blood donors in Hokkaido was 0·01% (56/432,167) and 3·9%, respectively [11]. These reports also suggested that a small but significant proportion of blood donors in Japan with or without elevated alanine aminotransferase (ALT) levels are viremic and are potentially able to cause transfusion-associated hepatitis E. Note that anti-HEV IgG and HEV levels in pooled plasma have not been reported yet. Thus, these data may indicate the need for precautions against the potential risk of transfusion-transmitted HEV infection, as previously discussed [12]. In addition to foods, the safety of plasma-derived products with respect to HEV may be an important issue and each product should be evaluated for safety against HEV contamination.

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

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

Materials and methods

Viral isolates

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

Table 1 Details of viral isolates used

Genotype[a]	Isolation ID	Viral titre: HEV genome[b]	Viral titre: HEV infectivity[c]	Used for
3_{JP-a}	swJB-N2	6·3	3·8	Liquid-heating, nanofiltration
3_{US}	swJB-M5	7·2	4·8	Nanofiltration, dry-heating
	swJB-M8	8·4	5·3[d]	Liquid-heating
3_{SP}	swJB-E8	7·5	4·8	Dry-heating
	swJB-E10	7·7	5·8[e]	Liquid-heating, nano-filtration
4_{JP}	swJB-H1	7·0	–	Nanofiltration
	swJB-H1/H7	7·0/7·4	4·8[f]	Liquid-heating
	swJB-H7	7·4	3·2[d]	Liquid-heating
	swJB-H8	6·8	3·8	Liquid-heating
	swJB-H21[g]	7·2	3·8	Liquid-heating

[a]The genotypes and clusters of isolates were grouped as described by Takahashi *et al.* and Lu *et al.* [24,25].
[b]Genome amount is indicated by log copies per mL. For swJB-M, specific primer sets and probes '(sense primer F2: 5′-TCGTGTACAAACCGAGATTC-3′, anti-sense primer R2: 5′-GCCCGGCAATATTGTTCTA-3′, Probe Flu2: 5′-GATGCAACCCCGGCAGTTGGTTTTC-FITC-3′ and Probe LC2: 5′-LCRed640-GCCCTGAGGTACTCTGGAATCATCCTATCC-3′)' were designed and used. For the other isolates, the primer set and probe (HEB6, HE87 and FAMlabeled probe FHEB8) designed by Jothikumar *et al.* [26] were used.
[c]Infectivity titre is given as log dilution non-detectable end-point per mL.
[d]Mean titre of two ('three) independent experiments.
[e]Mixture of H1 and H7 used.
[f]This isolate is derived from faeces of an experimentally infected piglet.

Isolation and purification of virus

Faecal samples (10 g) were resuspended with 100 ml of phosphate-buffered saline (PBS) and centrifuged at 1600 *g* for 10 min and the supernatant retained. Pellets were resuspended in 50 ml of PBS and the suspension was centrifuged again under the same conditions. Resultant pellets were resuspended with 25 ml of PBS and the suspension was centrifuged again. All these three supernatants were pooled and were filtered using an AP filter (AP2504700, Millipore, Billerica, MA, USA). After centrifugation at 10 000 *g* for 30 min, the supernatant was filtered through four sequential filters (5·0 μm; SMWP04700, 1·2 μm; RAWP04700, 0·8 μm; AAWP04700, and finally 0·45 μm; HAWP04700, Millipore). Then polyethylene glycol (PEG) 6000 (Wako Pure Chemical Industries, Osaka, Japan) and sodium chloride up to final concentrations of 8% (w/v) and 2·4% (w/v), respectively, were added to the final filtrate. The solution was stirred for 10 min and incubated overnight at 4°C. The solution was centrifuged at 10 000 *g* for 30 min and the precipitate was resuspended with one-tenth the volume of the original solution of PBS prior to the addition of PEG. The solution was sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The resultant supernatant was filtered in two steps (0·45 μm; SLHV033RS and 0·22 μm; SLGV033RS, Millipore), and the filtrate was aliquoted and stored at −80°C as HEV stock. Isolated HEV samples were allocated an isolation ID and preparation lot number.

Hepatitis E virus stocks were further purified for filtration experiments. The viral stocks in PBS were treated with 1% (v/v) Tween-80 (Wako Pure Chemical Industries) and 0·3% (v/v) Tri-n-butyl Phosphate (TNBP, Sigma, St. Louis, MO, USA) for 1 h at 30°C and then the solutions were ultracentrifuged at 150 000 *g* for 3 h at 4°C. The precipitates were resuspended in PBS and subsequently sonicated and centrifuged at 4000 *g* for 15 min at 4°C. The supernatants were filtered by sequential 0·22 and 0·1 μm filtration [SLGV033RS (0·22 μm) and SLVV033RS (0·1 μm); Millipore] and the filtrate was aliquoted and stored at −80°C as purified HEV stock. In addition, HEV Genotype 3_{Sp} derived from the culture media of infected A549 cells was treated with detergent alone, as described above, and subsequently used for filtration experiments.

Quantitative HEV RNA assay for each isolate

The total HEV RNA in each sample was extracted using the RNeasy Mini Kit (cat. 74104; Qiagen GmbH, Hilden, Germany) and then quantified by polymerase chain reaction (PCR) using specific primers. The copy number of swJB-M was quantified using specified primers and probes set from the light cycler (LC) RNA Amplification Kit Hybridization Probes (Roche Diagnostics, Basel, Switzerland) and LC quick system 350S (Roche Diagnostic). The assay conditions were as follows: reagents; 4·0 μl of 5× LC reverse transcription (RT)-PCR Mix HybProbe (Roche Diagnostic), 3·2 μl of 25 mM $MgCl_2$, 2·0 μl of 5 pmol/μl primer F+R, 2·0 μl of 2 pmol/μl probe Flu+LC, 3·4 μl of water, 0·4 μl of LC RT-PCR enzyme mix and 5·0 μl of template (total 20 μl), and reaction; 55°C 10 min, 95°C 30 second, 45 cycles of 95°C 5 second, 60°C 15 second, 72°C 13 second and subsequently 40°C 30 second. The copy number of ORF3 for swJB-N, swJB-E and swJB-H (genotypes 3_{JPn}, 3_{Sp} and 4_{JP}) was also quantified using a QuantiTect Probe RT-PCR Kit (Qiagen) and Applied Biosystems 7500 (Applied Biosystems, Foster City, CA, USA). The assay conditions were as follows: reagents; 25 μl of 2× QuantiTect Probe RT-PCR Master Mix (Qiagen GmbH), 1·0 μl of 20 μM primer Mix, 0·5 μl of 10 μM Probe, 0·5 μl of QuantiTect RT Mix, 13·0 μl of water and 10 μl of template (total 50 μl), and reaction; 50°C 30 min, 95°C 15 min, 45 cycles of 95°C 15 second and 60°C 35 second.

Infectivity assay for HEV

Infectivity of HEV was assayed according to Huang *et al.* [13] with minor modifications. A549 cells (kindly provided by Dr Takaaki Nakaya, Research Institute for Microbial Diseases, Osaka University) were cultured in DMEM (cat. 11995-065, Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (cat. SH30071·03; Hyclone, Logan, UT, USA), 100 U/ml penicillin, 100 μg/ml streptomycin (cat. 15140-122, Invitrogen) and Insulin-Transferrin-Selenium-X (ITS-X) supplement (cat. 51500-056, Invitrogen) at 37°C in 5% CO_2 in air. The composition of the medium used for the viral assay was Dulbecco's modified Eagle's medium (DMEM) containing 2% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, ITS-X supplement and 30 mM $MgCl_2$ (cat. 135-00165, Wako Pure Chemical Industries) at 37°C in 5% CO_2 in air. For the infectivity assay, A549 cells were seeded in a 12-well microplate ($3·6 \times 10^5$ cells/ml, 2 ml/well). After an overnight culture, the cells were inoculated with serial 10-fold dilutions of the virus stock solution (0·3 ml/well). On day 7 of culture, HEV RNA in cultured cells was assayed using the HEV RNA assay method described above. The infectivity of each stock of isolate used was determined from the dilution end-point where no RNA was detected.

Heat sensitivity of HEV during liquid- and dry-heating

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

The HEV precipitates described above were also resuspended with a Fibrinogen solution containing 1·3% (w/v) trisodium citrate dihydrate and 2·0% (w/v) L-arginine hydrochloride as a stabilizer that was collected just before the dry-heating step in the manufacture of Fibrinogen HT-Wf (Benesis). The HEV solutions were aliquoted at 2·0 ml/vial and freeze-dried using an optimized freeze drying cycle (programme) for this product (freeze dry systems cat. 7948020 and 7934024, Labconco, Kansas City, MO, USA). The freeze-dried samples in the vials were closed under vacuum. The vials were then heated at 60 or 80°C in a drying oven (cat. DK43; Yamato Scientific, Tokyo, Japan) for 72 h. The heated samples were cooled quickly and stored at 4°C until the assaying. Residual infectivity was assayed as described above. In addition, the residual water content of mock-infected samples prepared using the same freeze drier programme and conditions without spiking with HEV were assayed using the loss on drying test method described previously [16].

Removal of HEV by nanofiltration

Hepatitis E virus stocks that were detergent-treated, as described above, were thawed, concentrated, if required, sonicated and filtered using 0·22 μm (0·22 μm; SLGV033RS, Millipore) and Bemberg Microporous Membrane (BMM) filter (Planova®-75N (72 ± 4 nm, 0·001 m^2); Asahi Kasei Medical, Tokyo, Japan) immediately prior to nanofiltration. The viral samples were subjected to nanofiltration using BMM-35N (35 ± 2 nm), -20N (19 ± 2 nm) and -15N (15 ± 2 nm; Asahi Kasei Medical) under conditions where 2-ml samples were applied to 10^{-5} m^2 filters with 50 kPa and dead end filtration. The quantities of HEV RNA before and after filtration were measured using the quantitative HEV RNA assay described above.

Results

Viral preparations

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

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

Heat sensitivity of HEV

The heat-inactivation kinetics of HEV isolates from four clusters including two genotypes during liquid-heating using 25% albumin and PBS at 60°C for 5 h was evaluated. All isolates in PBS were inactivated below the detectable infectivity limit within 30 min at 60°C and showed a rapid inactivation. The log reduction factor (LRF) of genotype 3_{JPn}, 3_{Sp}, 3_{US} and 4_{JP} was ≥ 2·7, ≥ 3·7, ≥ 3·7 and ≥ 2·4, respectively. In contrast, all HEV isolates in the 25% albumin solution showed heat resistance, and residual infectivity was detected even in the samples heated for 5 h and the LRF was 2·0, 2·0, 1·0 and ≥ 2·2, respectively (Fig. 1).

The heat-inactivation kinetics of Genotype 3_{US} and 3_{Sp} in fibrinogen during dry-heating was also evaluated. The water content of freeze-dried samples containing the two HEVs was < 0·3%. Residual infectivity was not detected with the LRF

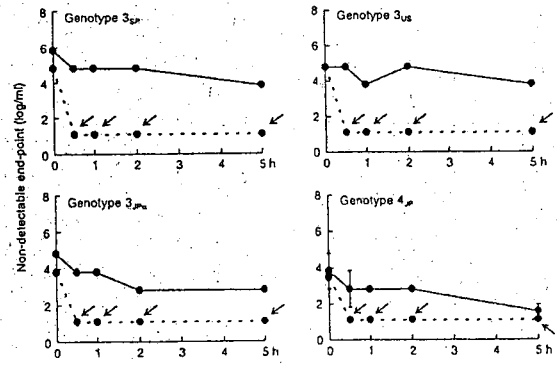

Fig. 1 Inactivation kinetics of the four HEV isolates during liquid-heating. Solid lines: HEV in 25% albumin. Broken lines: HEV in PBS. Arrow: infectious virus was not detected. Genotype 4_{JP}: $n = 3$.

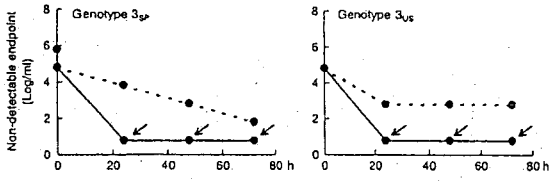

Fig. 2 Inactivation kinetics of the two HEV isolates during dry-heating. Solid lines: at 80°C. Broken lines: at 60°C. Arrow: infectious virus not detected.

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

	HEV[a]				
BMM-filtre	3_{JPN} (swJB-N2)	3_{US} (swJB-MS)	3_{SP} (swJB-E10)	3_{SP} (cultured HEV[d])	4_{JP} (swJB-H1)
BMM-35N (35 ± 2 nm)	(6·1/4·8)[b] 1·3[c]	(6·9/< 3·3) ≥ 3·6	(6·4/3·8) 2·6	(6·0/< 3·2) ≥ 2·8	(5·6/4·5) 1·1
BMM-20N (19 ± 2 nm)	(6·1/< 2·3) ≥ 3·8	(6·9/< 3·3) ≥ 3·6	(6·4/< 3·2) ≥ 3·2	(6·0/< 3·2) ≥ 2·8	(5·6/< 3·0) ≥ 2·6
BMM-15N (15 ± 2 nm)	(6·1/< 2·3) ≥ 3·8	(6·9/< 3·3) ≥ 3·6	(6·4/< 3·2) ≥ 3·2	(6·0/< 3·2) ≥ 2·8	(5·6/< 3·0) ≥ 2·6

[a]HEV is in PBS.
[b]Genome amount is indicated as total log copies. Left: before filtration; right: after filtration.
[c]Log reduction factor. Log reduction factor was calculated from the genome amount in the samples before and after filtration.
[d]Derived from cultured media of HEV-infected A549 cells.

≥ 4·0 after treatment at 80°C for 24 h in any samples. However, although the infectivity of HEV was reduced at an LRF of 2·0 and 3·0, respectively, residual infectivity was detected in all samples that were treated at 60°C for 72 h (Fig. 2). These results indicated that the heat sensitivity is different not by genotype or cluster, but by the composition of the sample.

Filtration of HEV

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

Discussion

Several reports suggested that some industrial swine farms and commercial swine livers in industrial as well as developing countries could be contaminated by HEV [4,9]. Yazaki *et al.* detected HEV genomes in commercial swine livers that had been eaten by a hepatitis E-infected patient, as shown by the identical sequences of HEV in the liver and patient's sample by genome analysis. They reported that the patient became infected by eating uncooked liver [4]. Our infection studies using piglets demonstrated that HEV was mainly detected in liver, intestines, serum and faeces, but not detected in muscles [17]. Current epidemiological studies revealed that the prevalence of HEV RNA or anti-HEV IgG-positive blood donors in Hokkaido and Tokyo was 0·01% (56/432,167) of RNA and 3·9% of IgG, and 0·01% (3/44,322) of RNA and 8·6% of IgG, respectively. In addition, the prevalence of anti-HEV IgG in Japan varies according to locality, 1·0–8·6% [11]. These results also suggest that although the possibility of transmission is not considered to be high at the moment, some patients who have HEV in their blood may donate blood and this could lead to a transfusion-transmitted infection. Consequently, a monitoring study for donated blood has been initiated in Hokkaido, Japan.

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

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

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

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

Acknowledgements

This study was conducted based on collaborative research projects involving Osaka University and Benesis Corporation, and Rakuno Gakuen University and Benesis Corporation. The authors thank Dr Andy Bailey, ViruSure GmbH for discussions and Dr Shoichi Ide, Asahi Kasei Medical Co., Ltd. for support and discussion regarding the Planova filtres.

References

1 Emerson SU, Anderson D, Arankalle A, Meng XJ, Purdy M, Schlauder GG, Tsarev SA: Hepevirus; in Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (eds): *Virus Taxonomy: Eighth Report of the International Committee on the Taxonomy of Viruses*. London, UK, Elsevier, 2005:853–857
2 Abe K, Li TC, Ding X, Win KM, Shrestha PK, Quang VX, Ngoc TT, Taltavull TC, Smirnov AV, Uchaikin VF, Luengrojanakul P, Gu H, El-Zayadi AR, Prince AM, Kikuchi K, Masaki N, Inui A, Sata T, Takeda N: International collaborative survey on epidemiology of hepatitis E virus in 11 countries. *Southeast Asian J Trop Med Public Health* 2006; 37:90–95
3 Meng XJ: Novel strains of hepatitis E virus identified from humans and other animal species: is hepatitis E a zoonosis? *J Hepatol* 2000; 33:842–845
4 Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, Okamoto H: Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. *J Gen Virol* 2003; 84:2351–2357
5 Li TC, Chijiwa K, Sera N, Ishibashi T, Etoh Y, Shinohara Y, Kurata Y, Ishida M, Sakamoto S, Takeda N, Miyamura T: Hepatitis E virus transmission from wild boar meat. *Emerg Infect Dis* 2005; 11:1958–1960
6 Takahashi K, Kitajima N, Abe N, Mishiro S: Complete or near-complete nucleotide sequences of hepatitis E virus genome recovered from a wild boar, a deer, and four patients who ate the deer. *Virology* 2004; 330:501–505
7 Matsubayashi K, Nagaoka Y, Sakata H, Sato S, Fukai K, Kato T, Takahashi K, Mishiro S, Imai M, Takeda N, Ikeda H: Transfusion-transmitted hepatitis E caused by apparently indigenous hepatitis E virus strain in Hokkaido, Japan. *Transfusion* 2004; 44:934–940
8 Caron M, Enouf V, Than SC, Dellamonica L, Buisson Y, Nicand E: Identification of genotype 1 hepatitis E virus in samples from swine in Cambodia. *J Clin Microbiol* 2006; 44:3440–3442
9 Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ: Detection and characterization of infectious Hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. *J Gen Virol* 2007; 88:912–917
10 Mizuo H, Yazaki Y, Sugawara K, Tsuda F, Takahashi M, Nishizawa T, Okamoto H: Possible risk factors for the transmission of hepatitis E virus and for the severe form of hepatitis E acquired locally in Hokkaido, Japan. *J Med Virol* 2005; 76:341–349
11 Ministry of Health, Labour and Welfare Japan: Conference documents of the subcommittee of safety control technologies on blood products of 1st conference in 2005. [In Japanese]. Available at http://www.mhlw.go.jp/shingi/2005/10/s1020-1.html
12 Fukada S, Sunaga J, Saito N, Fujimura K, Itoh Y, Sasaki M, Tsuda F, Takahashi M, Nishizawa T, Okamoto H: Prevalence of antibodies to hepatitis E virus among Japanese blood donors: identification of three blood donors infected with a genotype 3 hepatitis E virus. *J Med Virol* 2004; 73:554–561
13 Huang R, Li D, Wei S, Li Q, Yuan X, Geng L, Li X, Liu M: Cell culture of sporadic hepatitis E virus in China. *Clin Diagn Lab Immunol* 1999; 6:729–733
14 Emerson SU, Arankalle VA, Purcell RH: Thermal stability of hepatitis E virus. *J Infect Dis* 2005; 192:930–933
15 Tanaka T, Takahashi M, Kusano E, Okamoto H: Development and evaluation of an efficient cell-culture system for Hepatitis E virus. *J Gen Virol* 2007; 88:903–911
16 National Institute of Infectious Diseases Japan: *Test for Moisture Content in Minimum Requirements for Biological Products* 2006:280. Available at http://www.nih.go.jp/niid/MRBP/index-e.html
17 Hagiwara K, Iwabu Y, Kanai Y, Miyasho T, Daidoji T, Yunoki M, Tsujikawa M, Ohkubo Y, Yasue H, Ikuta K: Distribution and propagation of hepatitis E virus in experimentally infected swine. *The Open Veterinary Science Journal* 2007; 1:1–6
18 Roberts PL, Dunkerley C, McAuley A, Winkelman L: Effect of manufacturing process parameters on virus inactivation by dry heat treatment at 80°C in factor VIII. *Vox Sang* 2007; 92:56–63

19 Hattori S, Yunoki M, Tsujikawa M, Urayama T, Tachibana Y, Yamamoto I, Yamamoto S, Ikuta K: Variability of parvovirus B19 to inactivation by liquid heating in plasma products. *Vox Sang* 2007; 92:121–124

20 Yunoki M: Usefulness of Planova filtration in virus inactivation/removal. Planova workshop 2000 (Tokyo, Japan)

21 Yunoki M: Evaluation of the ability of Planova filtration to eliminate infectious pathogens. Planova workshop 2003 (Tokyo, Japan)

22 Kreil TR, Wieser A, Berting A, Spruth M, Medek C, Pölsler G, Gaida T, Hämmerle T, Teschner W, Schwarz HP, Barrett PN: Removal of small nonenveloped viruses by antibody-enhanced nanofiltration during the manufacture of plasma derivatives. *Transfusion* 2006; 46:1143–1151

23 Yokoyama T, Murai K, Murozuka T, Wakisaka A, Tanifuji M, Fujii N, Tomono T: Removal of small non-enveloped viruses by nanofiltration. *Vox Sang* 2004; 86:225–229

24 Takahashi M, Nishizawa T, Miyajima H, Gotanda Y, Iita T, Tsuda F, Okamoto H: Swine hepatitis E virus strains in Japan form four phylogenetic clusters comparable with those of Japanese isolates of human hepatitis E virus. *J Gen Virol* 2003; 84:851–862

25 Lu L, Li C, Hagedorn CH: Phylogenetic analysis of global hepatitis E virus sequences: genetic diversity, subtypes and zoonosis. *Rev Med Virol* 2006; 16:5–36

26 Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR: A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *J Virol Methods* 2006; 131:65–71

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No. 9

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
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況	Piron M, Vergés M, Muñoz J, Casamitjana N, Sanz S, Maymó RM, Hernández JM, Puig L, Portà s-M, Gascón J, Sauleda S. Transfusion. 2008 Sep; 48(9): 1862–8.	公表国	使用上の注意記載状況・その他参考事項等
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)				スペイン	人全血液-LR「日赤」 照射人全血液-LR「日赤」

研究報告の概要

○カタルーニャ(スペイン)の高リスク供血者における*Trypanosoma cruzi*感染の抗体陽性率

背景:ラテンアメリカ人のヨーロッパ(特にスペイン)移入が増加するにつれ、シャーガス病(中南米農村部に流行する人畜共通感染症)などの新たな病原体が報められるようになった。媒介生物サシガメがいない場合、非流行地域のシャーガス病伝播の主な形態のひとつは、輸血を介するものである。

研究デザインおよび方法:カタルーニャ血液銀行は、高リスク供血者におけるシャーガス病スクリーニング計画を実行し、供血者集団で*Trypanosoma cruzi*(*T. cruzi*)感染の血清学的検査陽性率を調査した。全検体にシャーガス抗体試験、ID-PaGIA (DiaMed社)とbioelisaシャーガス検査(Biokit社)の2種類の市販検査を使用した。

結果:全体の血清学的検査陽性率は0.62%であり、検査を実施した高リスク供血者1,770名のうち11名の陽性が確定した。最も陽性率が高かったのはボリビア出身の供血者であった(10.2%)。陽性供血者11名のうち1名は、シャーガス病流行地域に数年間滞在したことのあるスペイン人であった。さらに、陽性供血者の1名は、検出可能な寄生虫血症を呈した。

結論:本試験の結果は、非流行国の高リスク供血者に*T. cruzi*スクリーニング検査を実施する必要性を強調する。流行地域に居住経験のある(必ずしも当該地域で誕生することを意味するわけではない)高リスクに分類される人々を、検査対象に含めることが重要な知見として挙げられる。*T. cruzi*スクリーニングが全供血者に対してルーチンに実施されない場合には、供血前の問診における高リスク供血者の特定が重要である。

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
スペイン、カタルーニャの高リスク供血者集団における*T. cruzi*感染の抗体陽性率は0.62パーセントであったとの報告である。	日本赤十字社は、輸血感染症対策として献血時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、シャーガス病の既往がある場合には献血不適としている。日本在住の中南米出身献血者については、厚生労働科学研究「献血血液の安全性確保と安定供給のための新興感染症等に対する検査スクリーニング法等の開発と献血制限に関する研究」班と共同して検討する予定である。今後も引き続き情報の収集に努める。

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

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

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

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

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

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

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

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This study was funded in part by the Bayer Foundation and by grant 024/13/2004 from the Agència d'Avaluació de Tecnologies I Recerca Mèdiques (AATRM, Catalunya, Spain). CIBEREHD is funded by the Instituto de Salud Carlos III.

Received for publication January 18, 2008; revision received March 14, 2008, and accepted March 16, 2008.

doi: 10.1111/j.1537-2995.2008.01789.x

TRANSFUSION 2008;48:1862-1868.

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

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

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

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

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

MATERIALS AND METHODS

Donor selection and study design

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

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

Detection methods

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

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

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

Furthermore, all initially positive samples were assessed for the presence of parasite DNA in blood, using in-house real-time polymerase chain reaction (PCR).[11]

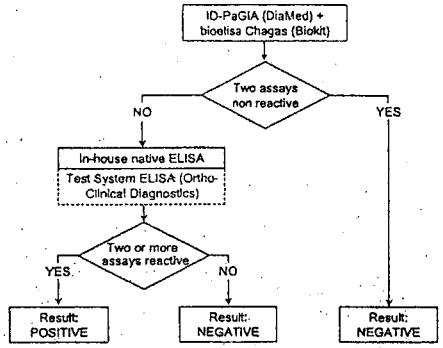

Fig. 1. Algorithm for *T. cruzi* serology interpretation.

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

RESULTS

Epidemiologic data

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

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

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

The most highly represented country of origin was Colombia, accounting for 22.3 percent of at-risk donors included in the study, followed by Argentina and Ecuador, accounting for 19.5 and 14.6 percent, respectively (Table 3). The majority of mothers of the 37 donors in Group 2 came from Argentina (10), followed by Colombia (7), Chile (7), and Peru (3). Most donors from Group 3 (n = 209) had visited various endemic countries during one or several trips.

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

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

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

DISCUSSION

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

In nonendemic countries, blood transfusion is one of the main modes of acquiring the infection, and cases of transmission before screening for *T. cruzi* infection became mandatory in blood donors have been reported in Spain.[17,18] European legislation requires permanent rejection of persons with a history of Chagas disease for blood donation.[19] Nevertheless, most people do not present any health problem until many years after acquiring the infection. Because of the increasing number of people from Latin America residing in Europe, and European people who reside for a time in an endemic area, implementation of screening programs for this disease in at-risk donors may be advisable in all European blood banks.

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

TABLE 1. Epidemiologic data of donors included in the study

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

* Data are reported as number (%).
† Data are reported as mean (SD).

TABLE 2. Living conditions in endemic area

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

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

Country	Tested for anti-*T. cruzi**	Percentage of official immigrant population in Catalonia	Number	Anti-*T. cruzi*–positive donors Rate by country (%)
Colombia	340 (22.3)	13.8		
Argentina	298 (19.5)	11.7	2	2/298 (0.67)
Ecuador	223 (14.6)	28.2	1	1/223 (0.45)
Uruguay	127 (8.3)	4.4		
Peru	123 (8.1)	8.9		
Brazil	113 (7.4)	3.9		
Venezuela	86 (5.6)	2.4		
Chile	77 (5.0)	4.2		
Bolivia	59 (3.9)	8	6	6/59 (10.2)
Mexico	40 (2.6)	2.6		
Paraguay	15 (1.0)	1.1	1	1/15 (6.7)
Honduras	10 (0.7)	1.3		
El Salvador	6 (0.4)	0.4		
Nicaragua	3 (0.2)	0.1		
Costa Rica	2 (0.1)	0.1		
Guatemala	1 (<0.1)	0.1		
Panama	1 (<0.1)	0.1		
Total	1524		10	

* Data are reported as number (%).

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

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

* All initially reactive results were confirmed as positive or negative by in-house native ELISA. Cohen's kappa index, 0.471.[31]
† In-house native ELISA result positive.
‡ In-house native ELISA result negative.

TABLE 5. Epidemiologic data of the 11 positive donors

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

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

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

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

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

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

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

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

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

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

In summary, this study reports a seroprevalence of *T. cruzi* infection of 0.62 percent among at-risk donors in Catalonia and emphasizes the need to include individuals who have resided in, but were not necessarily born in endemic areas as at-risk donors. The difficulty of this type of selective screening is proper identification of the risk population, which essentially depends on the predonation interview. Latin Americans accounted for more than 1 percent of the total of donors in our study, and this substantial contribution underscores the need to accept them as donors.

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

ACKNOWLEDGMENTS

We are grateful to the Departament de Salut de la Generalitat de Catalunya for its support. We thank Celine Cavallo for English revision.

REFERENCES

1. Senior K. Chagas disease: moving towards global elimination. Lancet Infect Dis 2007;7:572.
2. Prata A. Clinical and epidemiological aspects of Chagas' disease. Lancet Infect Dis 2001;1:92-100.
3. Barrett MP, Burchmore RJ, Stich A, Lazzari JO, Frasch AC, Cazzulo JJ, Krishna S. The trypanosomiases. Lancet 2003; 362:1469-80.
4. Punukollu G, Gowda RM, Khan IA, Navarro VS, Vasavada BC. Clinical aspects of the Chagas heart disease. Int J Cardiol 2007;115:279-83.
5. World Health Organization. Control of Chagas' disease. Second report of the WHO Expert Committee. World Health Organ Tech Rep Ser 2002;905:i-vi, 1-109.
6. Pedreira de Freitas JL, Amato Neto V, Sonntag R, Biancalana A, Nussenszweig V, Barreto JG. Primeiras verificacões de transmissão acidental da moléstia de Chagas ao homem por transfusão de sangue. Rev Paul Med 1952;40: 36-40.
7. Dias JC, Silveira AC, Schofield CJ. The impact of Chagas' disease control in Latin America: a review. Mem Inst Oswaldo Cruz 2002;97:603-12.
8. Instituto Nacional de Estadistica. Spain census data [database on the Internet]. Madrid: INE; 2008 [cited 2007 Jan]. Available from: http://www.ine.es
9. Gascon J. Diagnóstico y tratamiento de la enfermedad de Chagas importada. Medicina Clinica (Barcelona) 2005;125: 230-5.
10. Riera C, Guarro A, Kassab HE, Jorba JM, Castro M, Angrill R, Gállego M, Fisa R, Martin C, Lobato A, Portús M. Congenital transmission of *Trypanosoma cruzi* in Europe (Spain): a case report. Am J Trop Med Hyg 2006;75:1078-81.

11. Muñoz J, Portus M, Corachan M, Fumado V, Gascon J. Congenital *Trypanosoma cruzi* infection in a non-endemic area. Trans R Soc Trop Med Hyg 2007;101:1161-2.
12. Young C, Losikoff P, Chawla A, Glasser L, Forman E. Transfusion-acquired *Trypanosoma cruzi* infection. Transfusion 2007;47:540-4.
13. Real Decreto 1088/2005 por el que se establecen los requisitos técnicos y condiciones mínimas de la hemodonación y de los centros y servicios de transfusión del 20 de septiembre 2005, núm.225, 31288-31304.
14. Piron M, Fisa R, Casamitjana N, López-Chejade P, Puig L, Vergés M, Gascón J, Gómez i Prat J, Portús M, Sauleda S. Development of a real-time PCR assay for *Trypanosoma cruzi* detection in blood samples. Acta Tropica 2007;103:195-200.
15. Schmunis GA, Cruz JR. Safety of the blood supply in Latin America. Clin Microbiol Rev 2005;18:12-29.
16. Kirchhoff LV, Paredes P, Lomelí-Guerrero A, Paredes-Espinoza M, Ron-Guerrero CS, Delgado-Mejía M, Peña-Muñoz JG. Transfusion-associated Chagas' disease (American trypanosomiasis) in Mexico: implications for transfusion medicine in the United States. Transfusion 2006;46:298-304.
17. Abalo M, Adelantado M, Areal C, Castrillo A, Castro A, Cid J, Eiras A, Flores J, Cabrera J. Tracing of one year of Chagas screening at the Centro de Transfusión de Galicia (C.T.G.) concerning a positive blood donor. Abstract. XVIIth Regional Congress of the ISBT, Europe. Madrid June 23-27, 2007. Vox Sang 2007;93 Suppl. 1:140.
18. Forés R, Sanjuán I, Portero F, Ruiz E, Regidor C, López-Vélez R, Linares M, Gil S, Ojeda E, Krsnik I, Bautista G, Vallejo C, García-Marco J, Fernández MN, Cabrera JR. Chagas disease in a recipient of cord blood transplantation. Bone Marrow Transplant 2007;39:127-8.
19. Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components.
20. Steele LS, MacPherson DW, Kim J, Keystone JS, Gushulak BD. The sero-prevalence of antibodies to *Trypanosoma cruzi* in Latin American refugees and immigrants to Canada. J Immigr Minor Health 2007;9:43-7.
21. Frank M, Hegenscheid B, Janitschke K, Wienke T. Prevalence and epidemiological significance of *Trypanosoma cruzi* infection among Latin American immigrants in Berlin, Germany. Infection 1997;25:355-8.
22. Centers for Disease Control and Prevention (CDC). Blood donor screening for Chagas' disease United States, 2006-2007. MMWR Morb Mortal Wkly Rep 2007;56:141-3.
23. Tobler LH, Contestable P, Pitina L, Groth H, Shaffer S, Blackburn GR, Warren H, Lee SR, Busch MP. Evaluation of a new enzyme-linked immunoabsorbent assay for detection of Chagas antibody in US blood donors. Transfusion 2007;47:90-6.
24. Leiby DA, Herron RM, Read EJ, Lenes BA, Stumpf RJ. *Trypanosoma cruzi* in Los Angeles and Miami blood donors: impact of evolving donor demographics on seroprevalence and implications for transfusion transmission. Transfusion 2002;42:549-55.
25. Algora M, Torres P, Ariza B, Moreno R, Herrero ML, Martínez MA, Ruíz M, Zuazagoitia I, Quesada S, Fernández M, García-Carpio R, Zancada G, Corredor E, Barbolla L. Seroprevalence of *Trypanosoma cruzi* antibodies in blood donors in Madrid area. Abstract. XVIIth Regional Congress of the ISBT, Europe. Madrid June 23-27, 2007. Vox Sang 2007;93 Suppl 1:140.
26. Ontañon A, Arroyo JL, Romón I, Amunarriz C, Hermosa V. Evaluation of a strategy for *Trypanosoma cruzi* screening and its impact on blood donation. Abstract. XVIIth Regional Congress of the ISBT, Europe. Madrid June 23-27, 2007. Vox Sang 2007;93 Suppl 1:142.
27. Castro E. Transfusión sanguínea y enfermedad de Chagas: iniciativas en Centros de Transfusión de España. Enf. Emerg 2006;8 Suppl 1:48-50.
28. Assal A, Aznar C. Chagas' disease screening in the French blood donor population. Screening assays and donor selection. Enf. Emerg 2007;9 Suppl 1:36-40.
29. Gironés N, Bueno JL, Carrión J, Fresno M, Castro E. The efficacy of photochemical treatment with methylene blue and light for the reduction of *Trypanosoma cruzi* in infected plasma. Vox Sang 2006;91:285-91.
30. Castro E, Gironés N, Bueno JL, Carrión J, Lin L, Fresno M. The efficacy of photochemical treatment with amotosalen HCl and ultraviolet A (INTERCEPT) for inactivation of *Trypanosoma cruzi* in pooled buffy-coat platelets. Transfusion 2007;47:434-41.
31. Cohen JA. Coefficient of agreement for nominal scales. Educ Psychol Meas 1960;20:37-46.

別紙様式第2-1

No. 33

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
				2008. 10. 15	該当なし	
一般的名称	人全血液		研究報告の公表状況	BuaNews online, Mon 13 Oct 2008. available at http://www.buanews.gov.za/news/08/08101311151006	公表国	
販売名（企業名）	人全血液-LR「日赤」（日本赤十字社） 照射人全血液-LR「日赤」（日本赤十字社）				南アフリカ	

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
○アレナウイルスと特定された未知の疾患 南アフリカ、ヨハネスブルグで3名の死者を出したウイルスは、暫定的に西アフリカのラッサウイルスに近い、齧歯類媒介性アレナウイルスであると特定された。ウイルスは感染マウスの排泄物を介し、人間の食物やハウスダストを汚染する可能性がある。 南アフリカ国立感染症研究所（NICD）と保健省は共同で、このウイルスが体液を介してヒトからヒトに感染するため、「患者の看護に特別な予防的措置が必要である」との声明を発表した。3名の死因を確定するには更なる検査が必要である。新たなアレナウイルスであるかどうか、ならびに当該ウイルスの分布について検討を行う必要がある。ヒトに疾患を引き起こすアレナウイルスが南アフリカの齧歯類に存在することはまだ示されていないとNICDは述べた。 1人目の女性患者は、9月中旬に重篤な容態でザンビアから搬送され、Morningside Medi-Clinicに入院し、2日後に死亡した。約2週間後、1人目の患者の搬送に同行した救急救命士が死亡し、間もなく看護師が死亡した。 1人目の患者と接触した他の3名の患者は退院したことが確認されているが、依然として2名が厳重な監視下に置かれている。1名は、発熱およびインフルエンザ様症状を発症した救急救命士であり、もう1名は2人目の患者をケアした女性看護師である。彼女は、隔離され抗ウイルス剤ribavirinの投与を受けており、現在は安定している。	人全血液-LR「日赤」 照射人全血液-LR「日赤」 血液を介するウイルス、細菌、原虫等の感染 vCJD等の伝播のリスク

報告企業の意見	今後の対応
南アフリカ、ヨハネスブルグで3名の死者を出したウイルスは、暫定的に西アフリカのラッサウイルスに近い、齧歯類媒介性アレナウイルスであると特定されたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国（入国）後4週間は献血不適としている。今後も引き続き、新興・再興感染症の発生状況等に関する情報の収集に努める。

BuaNews ONLINE
www.buanews.gov.za

Compiled by the Government Communication and Information System
Date: 13 Oct 2008
Title: Unknown illness identified as Arenavirus

By Luyanda Makapela

Johannesburg - The virus which has caused the death of three people has been provisionally identified as the rodent-borne Arenavirus.

The Arenavirus, related to the Lassa Fever Virus of West Africa, causes chronic infections in multimammate mice. Infected mice's excretion contains the virus which can contaminate human food or house dust.

A joint statement by the National Institute for Communicable Disease (NICD) and the Department of Health explained that the Arenavirus is a disease spread from human to human through the contact of body fluids.

"Special precautions are required in nursing patients," a statement said.

The finding follows blood samples being sent to Atlanta, in the United States to determine the cause of the deaths of three people who had been suspected of contracting Viral Haemorrhagic Fever.

The virus is similar to Lassa Fever, the department said. It has previously been found in rodents elsewhere in Africa, but has not been found to cause disease in humans other than in West Africa.

Further tests are needed to confirm the diagnosis by growing the virus in culture.

"It needs to be determined whether it is a previously unrecognised member of the Areaviruses, and what its distribution is. There is no indication as yet that Arenaviruses which cause disease in humans are present in South African rodents," the NICD said.

The first victim, who had to be flown in from Zambia in a critical condition, was admitted to the Morningside Medi-Clinic in mid September. She died two days later.

About two weeks later, the paramedic who had flown in with the first victim, was admitted at the same clinic presenting the same symptoms.

A nurse, Gladys Mthembu died shortly afterwards. According to certain reports Ms Mthembu's family has been given a go-ahead to continue with the funeral arrangements as her bedroom had been cordoned off by health officials

Maria Mokubung, a cleaner at the Morningside Medi-Clinic, who also died last weekend has since been ruled out as a possible victim of the virus

Meanwhile the Gauteng Health Department has confirmed that the three other patients, including nurse's female supervisor, who had been under observation for showing symptoms of the virus have been discharged.

They had been in contact with the nurse who died.

However, departmental spokesperson Phumelele Kaunda said there were two contacts that were still under active surveillance after being admitted for observation.

The one patient is a paramedic who had contact with the first patient and developed fever and flu-like symptoms. He was admitted initially in Flora Clinic and then transferred to Morningside Medi-Clinic with a diagnosis of kidney stones.

The other patient is a nurse who attended to the second patient and developed signs and symptoms similar to the first three patients. She is being treated in isolation and received the anti-viral medication, ribavirin. The patient is presently stable.

Gauteng Health MEC Brian Hlongwa meanwhile has sent condolences to the families of those that were killed by the viral infection, particularly families of health professionals who died in the line of duty.

"This illustrates the dedication of our health professionals and the need to society to respect and honour the work that they do," said MEC Hlongwa.

He also thanked the NICD, the National Health Laboratory Service, Centre for Disease Control in Atlanta and the World Health Organisation for ensuring that the results were made available soon. - BuaNews

医薬品　研究報告　調査報告書

識別番号・報告回数			報告日	第一報入手日 2008年10月20日	新医薬品等の区分 該当なし	総合機構処理欄
一般的名称	別紙のとおり		研究報告の公表状況	ProMED-mail, 20081028.3409	公表国 ザンビア・南アフリカ	
販売名（企業名）	別紙のとおり					

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
問題点：南アフリカにおいて、アレナウイルス科の新たなウイルスによる見られる感染により5人の患者が報告された。 初発患者(症例1)の発症は9/2日で、これに続いて3人の二次感染症例と1人の三次感染患者が報告された。初発患者と二次感染の3人は死亡し、三次感染症例は現在入院中である。患者の年齢層は33～47才、女性4人と男性1人。初発患者の感染源は判っていない。他の4人の患者は全員が医療施設内で、初発患者もしくは二次感染患者の血液・体液と接触があった可能性があった。初発患者はザンビア在住で、治療のための南アフリカへの移送後に死亡した。症例2は、症例1の移送に付き添った救急隊員の1人で、症例3は集中治療室にいた症例1の看護を担当していた。症例4は症例1が入院していた部屋の清掃を行った。症例5は症例2の看護を担当した。二次および三次感染患者の潜伏期間は7～13日と考えられている。死亡した4人の患者の発病から死亡までの期間は9～12日であった。患者全員が初発症状として発熱・筋肉痛・頭痛を伴うインフルエンザ様症状を示した。7日間で重症度が増し、いずれも下痢と咽頭痛が見られた。第6～8病日に顔面と躯幹の麻疹様発疹が報告されている。3人に顔面の浮腫があった。死亡した患者では、末期症状として呼吸困難・神経学的症状・循環不全を伴う突然で急速な状態の悪化が見られた。出血症状は著明な特徴ではないが、1人に皮下出血、もう1人は穿刺部位からの持続出血が見られた。暫定的な検査により、今回の感染はアレナウイルス科における新たな異なるウイルスと見られている。 現在(10/28日)まで新たな感染疑い症例は発生していない。感染流行は封じ込められたようであり、医療施設内環境下で濃厚接触者だけに感染が限定されている。病原体の詳細な特徴については、現在調査中であり、初発患者の感染源についての調査も必要である。症候性感染発生の可能性の検討も、感染流行の範囲や臨床像をより理解するために重要である。	記載なし

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

MedDRA/J ver.11.1

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人免疫グロブリン、⑤乾燥ペプシン処理人免疫グロブリン、⑥乾燥スルホ化人免疫グロブリン、⑦乾燥スルホ化人免疫グロブリン*、⑧乾燥濃縮人活性化プロテインC、⑨乾燥濃縮人血液凝固第Ⅷ因子、⑩乾燥濃縮人血液凝固第Ⅸ因子、⑪乾燥抗破傷風人免疫グロブリン、⑫抗HBs人免疫グロブリン、⑬トロンビン、⑭フィブリノゲン加第ⅩⅢ因子、⑮乾燥濃縮人アンチトロンビンⅢ、⑯ヒスタミン加人免疫グロブリン製剤、⑰人血清アルブミン*、⑱人血清アルブミン*、⑲乾燥ペプシン処理人免疫グロブリン*、⑳乾燥人血液凝固第Ⅸ因子複合体*、㉑乾燥濃縮人アンチトロンビンⅢ
販売名（企業名）	①献血アルブミン20“化血研”、②献血アルブミン25“化血研”、③人血清アルブミン“化血研”*、④“化血研”ガンマーグロブリン、⑤献血静注グロブリン“化血研”、⑥献血ベニロンーⅠ、⑦ベニロン*、⑧注射用アナクトC2,500単位、⑨コンファクトF、⑩ノバクトM、⑪テタノセーラ、⑫ヘパトセーラ、⑬トロンビン“化血研”、⑭ボルヒール、⑮アンスロビンP、⑯ヒスタグロビン、⑰アルブミン20%化血研*、⑱アルブミン5%化血研*、⑲静注グロブリン*、⑳ノバクトF*、㉑アンスロビンP1500注射用
報告企業の意見	アレナウイルス属は、エンベロープをもつ1本鎖RNA(－)ウイルスである。齧歯類に寄生し、慢性腎臓感染をおこす。齧歯類の尿中は高ウイルス価であり、ヒトの食品やハウスダストを汚染する。曝露したヒトは偶発的宿主となる。このウイルスの原型はリンパ球性脈絡膜髄膜炎ウイルス（LCMV）であり、ヒトに感染するとインフルエンザ様症状、無菌性髄膜炎もしくは重症髄膜脳炎を発症する。出血熱症候群の原因となるArenaviruses は南米(New World arenaviruses)から数多く報告されている。いわゆるOld World arenaviruses は世界中に分布するLCMVと、西アフリカのナイジェリア、シエラレオネ、リベリア、ギニアを中心に1年間に最大50万人が感染し、実際にはさらに広い地域に分布すると見られているラッサ熱ウイルスである。ラッサ熱ウイルス感染の臨床症状としては、不顕性、軽症発熱性疾患から劇症出血性疾患まで様々であり、致死率は一般的な社会環境における1～2%から、入院患者では20%、院内感染では40%以上に及ぶこともある。西アフリカ一帯に生息する野ネズミの一種であるマストミス（*Mastomys natalensis*）は、ラッサ熱ウイルスの最重要宿主であり、その分布は、西アフリカから東アフリカ一帯と、南アフリカ北東端まで南に広がっている。他のMastomys種とも分布域が重複し、アレナウイルスは過去にはアフリカ南部の齧歯類でも確認されている。 (http://www.forth.go.jp/cgi-bin/promed/search.cgi?title_link=20081029-0050&button_detail=on) 弊所の血漿分画製剤の製造工程には、冷エタノール分画工程、ウイルス除去膜ろ過工程あるいは加熱工程等の原理の異なるウイルス除去及び不活化工程が存在しているので、ウイルスクリアランスが期待される。 各製造工程のウイルス除去・不活化効果は、「血漿分画製剤のウイルスに対する安全性確保に関するガイドライン（医薬発第1047号、平成11年8月30日）」に従い、ウシウイルス性下痢ウイルス（BVDV）、仮性狂犬病ウイルス（PRV）、ブタパルボウイルス（PPV）、A型肝炎ウイルス（HAV）または脳心筋炎ウイルス（EMCV）をモデルウイルスとして、ウイルスプロセスバリデーションを実施し、評価を行っている。今回報告したアレナウイルス属は、エンベロープの有無、核酸の種類等からモデルウイルスとしてはBVDVが該当すると考えられるが、上記バリデーションの結果から、BVDVの除去・不活化効果を有することを確認している。 また、これまでに当該製剤によるアレナウイルス感染の報告例は無い。 以上の点から、当該製剤はアレナウイルスに対する安全性を確保していると考える。

*現在製造を行っていない

Archive Number 20081028.3409
Published Date 28-OCT-2008
Subject PRO/AH/EDR> Undiagnosed fatalities - S. Africa ex Zambia (10): arenavirus

UNDIAGNOSED FATALITIES - SOUTH AFRICA ex ZAMBIA (10): ARENAVIRUS
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A ProMED-mail post
<http://www.promedmail.org>
ProMED-mail is a program of the
International Society for Infectious Diseases
<http://www.isid.org>

Date: Fri 24 Oct 2008
Source: Communicable Diseases Communique Vol 7, No 10, Oct 2008 [edited]
<http://www.nicd.ac.za/pubs/communique/2008/NICDCommOct08Vol07_10.pdf>

Arena virus outbreak, South Africa — Update

This updates all previous reports and includes available data as of 24 Oct 2008. An outbreak of infection due to an arenavirus was identified in South Africa in early October 2008. A total of 5 cases has been reported for the period 12 Sep to 24 Oct 2008.

The primary case (case 1) had onset of illness on 2 Sep 2008. An additional 3 secondary cases (case 2, 3 and 4) and 1 tertiary case (case 5) have been confirmed to have an arenavirus infection by laboratory testing. The primary case and 3 secondary cases have died. The tertiary case is currently hospitalized. Ages of cases ranged from 33 to 47 years. 4 cases were female and 1 male. The source of infection is, as yet, unknown for the primary case. The other 4 cases all had potential exposure to blood and/or body fluids of a primary or secondary case in the health-care setting.

The primary case was a safari booking agent resident in Zambia. The patient was flown to South Africa for medical care in a critically ill condition on 12 Sep 2008, and died on 14 Sep 2008. Case 2 was a paramedic who cared for case 1 during the transfer from Zambia on 12 Sep 2008 and case 3 was a nurse who cared for case 1 in the intensive care unit from 12-14 Sep 2008. Case 2 was admitted on 27 Sep 2008 and died on 2 Oct 2008 and case 3 was admitted on 30 Sep 2008 and died on 5 Oct 2008. On 14 Sep 2008, case 4 performed terminal cleaning of the room in which case 1 was hospitalized. The 5th patient is a nurse who cared for case 2 from 27 Sep 2008 to 2 Oct 2008. She became ill on 9 Oct 2008 and is currently critical but stable. Ribavirin has been used for treatment in this case based on good evidence of efficacy in patients with Lassa fever (an arenavirus infection). The estimated incubation period (interval from exposure to symptom onset) in secondary and tertiary cases ranges from 7 to 13 days. In 4 patients who died, the interval from onset of illness to death ranged from 9 to 12 days (Figure 1).

Only limited clinical data are currently available for case 4, who presented late in the course of illness with bleeding and confusion and died soon thereafter. Clinical features of the remaining 4 cases, for which more clinical data were available, are presented. All patients presented initially with a non-specific flu-like illness with symptoms of fever,headache and myalgia. The illness increased in severity over 7 days with all 4 patients developing diarrhoea and pharyngitis during the course of illness. A morbiliform rash on the face and trunk was reported in 4 cases on day 6 - 8 of illness. Facial swelling occurred in 3 patients. There appeared to be an initial clinical improvement after hospital admission in 3 patients, followed by clinical deterioration. Sudden and rapid deterioration

with respiratory distress, neurological signs and circulatory collapse were terminal features in all patients who died. Bleeding was not a prominent feature. However, one patient had a petechial rash and another had oozing of blood from venepuncture sites. Chest pain was reported in case 1.

At the time of admission all patients had thrombocytopenia (range: 42-104 X109/L). Liver transaminases (AST and ALT) were available for 4 of 5 cases and were variable at the time of admission, however all 4 patients had raised AST and ALT during the course of their illness. Leucopenia was present on admission in 2 patients and 3 patients had a normal white blood cell count on admission. 4 patients subsequently developed leucocytosis during the course of hospitalisation. All contacts (family members, friends and healthcare staff) are being monitored with twice daily temperature measurements for a period of 21 days after the last exposure to a known case. In addition, safe burial of the deceased has been supervised by environmental health officers. Full personal protective equipment (PPE) and isolation precautions as per VHF protocols have been instituted.

The causative agent in this outbreak was initially identified as an Old World arenavirus by immunohistochemical tests performed at the Infectious Diseases Pathology Branch of the Centers for Disease Control and Prevention in Atlanta, USA, and on autopsy liver and skin samples taken with biopsy needles and skin punches in the Special Pathogens Unit of the National Institute for Communicable Diseases, National Health Laboratory Service, Sandringham (SPU-NICD/ NHLS), South Africa, from cases 2 and 3 on 9 Oct 2008 under biosafety level 4 laboratory conditions. Subsequently, infection with an Old World arenavirus has been confirmed in all 5 cases by positive PCR results and virus isolation by SPUNICD/ NHLS and CDC. Analysis of sequencing data generated at SPU-NICD/NHLS, Columbia University, New York, and CDC, Atlanta appears to indicate that the current outbreak is caused by a unique Old World arenavirus.

There are currently no additional suspected cases. The outbreak appears to be contained and has been confined to individuals with very close contact in a health-care setting. Monitoring of contacts, active case finding and investigation and management of suspected cases will continue as needed. Further characterization of the causative agent is under way and investigation into the source of infection in the primary case is required. Additional studies to determine whether mild/asymptomatic infection occurred amongst close contacts and other exposed individuals would be essential in better characterizing the extent of this outbreak and clinical spectrum of disease.

Arenaviruses are a family of enveloped negative sense single-stranded RNA viruses. Members of the family are parasites of rodents, in which they establish chronic renal infection. High titres of virus are present in rodent urine, which can contaminate human food or house dust. Exposed humans may become infected as accidental hosts. The prototype of the family is lymphocytic choriomeningitis (LCM) virus and infection of humans with this virus may present as an influenza-like illness, aseptic meningitis or severe meningo-encephalomyelitis. Arenaviruses which cause a haemorrhagic fever syndrome are well documented in South America (New World arenaviruses, including Junin, Machupo, Sabia and Guanarito viruses). The so-called Old World arenaviruses include LCM which in fact has a worldwide distribution, and Lassa fever virus which affects up to 500 000 people annually in West Africa, specifically in Nigeria, Sierra Leone, Liberia and Guinea, but the virus is suspected to be more widely distributed in that region.

The clinical spectrum of Lassa fever virus infection ranges from inapparent, through mild febrile illness to fulminant haemorrhagic disease, and mortality rates vary from 1-2 percent among cases in the community at large, through 20 percent among hospitalized patients, to >40 percent in nosocomial outbreaks. The multimammate mouse (Mastomys natalensis), which is the most important host of Lassa fever virus, has a distribution extending from West Africa across to East Africa and from there southwards to the northeastern corner of South Africa. Its distribution overlaps with that of other Mastomys species, and arenaviruses have been found in southern African rodents in the past, but there has been no previous association of these viruses with human disease despite sustained monitoring. Preliminary

testing indicates that the virus associated with the present nosocomial disease outbreak is a distinct new member of the family.

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[This update provides a definitive account of the recent outbreak of arenavirus-associated disease in South Africa. A primary case (case 1) had onset of illness on 2 Sep 2008. An additional 3 secondary cases (case 2, 3 and 4) and 1 tertiary case (case 5) have been confirmed to have an arenavirus infection by laboratory testing. Case 5 (not previously reported) is a nurse who cared for case 2 from 27 Sep 2008 to 2 Oct 2008. She became ill on 9 Oct 2008 and is currently critical but stable. Cases 1, 2, 3 and 4 did not survive infection.

Infection with an Old World arenavirus has been confirmed in all 5 cases by positive PCR results and virus isolation by SPUNICD/ NHLS and CDC. Analysis of sequencing data generated at SPU-NICD/NHLS, Columbia University, New York, and CDC, Atlanta, appears to indicate that the current outbreak is caused by a unique Old World arenavirus.

There are currently no additional suspected cases. The outbreak appears to be contained and has been confined to individuals with very close contact in a health-care setting. Monitoring of contacts, active case finding and investigation and management of suspected cases are continuing. Further characterization of the causative agent is under way, as is investigation into the source of infection in the primary case.
- Mod.CP]

[see also:
Undiagnosed fatalities - S. Africa ex Zambia (09): arenavirus 20081018.3300
Undiagnosed fatalities - S. Africa ex Zambia (08): arenavirus 20081013.3241
Undiagnosed fatalities - S. Africa ex Zambia (07): arenavirus 20081012.3234
Undiagnosed fatalities - South Africa ex Zambia (06): WHO 20081010.3211
Undiagnosed fatalities - South Africa ex Zambia (05) 20081008.3192
Undiagnosed fatalities - South Africa ex Zambia (04) 20081008.3188
Undiagnosed fatalities - South Africa ex Zambia (03) 20081007.3178
Undiagnosed fatalities - South Africa ex Zambia (02) 20081006.3157
Undiagnosed fatalities - South Africa ex Zambia: RFI 20081005.3139]
.....cp/ejp/dk

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別紙様式第2-1

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 10. 17	該当なし	
一般的名称	人全血液		研究報告の公表状況		公表国	
販売名(企業名)	人全血液-LR「日赤」(日本赤十字社) 照射人全血液-LR「日赤」(日本赤十字社)			ABC Newsletter, No. 38. 2008 Oct 17.	イタリア	

研究報告の概要

○イタリアで久々に発生したWNV症例

2008年、イタリアで久々にヒトのウエストナイルウイルス(WNV)脳炎が2例報告された。

1例目は、最近ウマ(6例)のWNV確定症例およびトリ(13例)のWNV陽性が特定されているフェラーラとボローニャの間に位置する農村地帯在住の80歳代の女性患者である。患者に渡航歴はなく、9月5日に発熱および複数回の嘔吐を発症した後、高熱、嘔吐、意識障害、幻覚を呈し、9月19日にイモラの病院に入院したが救急室で痙攣状態となった。その後回復したが、ELISAによるWNV特異抗体検査で急性WNV感染が示され、さらに追加検査によりWNV特異抗体が確認された。10月9日のユーロサーベイランスレポートは、検査結果はWNVに対する抗体反応であり、WNV神経侵襲性感染の仮説を裏づけると述べている。患者の家から2、3km以内の場所には、数種類の鳥類集団が生息し、蚊(イエカ、ヒトスジシマカ)が発生している大きな沼がある。

神経浸襲性WNV疾患の2例目は、フェラーラ在住の60歳代後半の男性で、10月3日にボローニャで特定された。患者は、高熱を伴う急性髄膜脳炎の症状を発現し、血清および脳脊髄液検体はWNV特異IgG、IgM抗体陽性で、2回の血清RT-PCR検査は陽性だった。

WNV髄膜脳炎の積極的サーベイランスプログラムが開始され、当該地域で供血者スクリーニング用核酸増幅検査が導入された。また、イタリアの国立血液センターは、全血液センターに対し、当該地域に1日以上滞在したことのある供血者を28日間供血延期とするように指導した。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2008年、イタリアで久々にヒトのウエストナイルウイルス(WNV)脳炎が2例報告されたため、WNV髄膜脳炎の積極的サーベイランスプログラムが開始され、供血者スクリーニング用核酸増幅検査の導入、28日間供血延期措置がとられたとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、ウエストナイルウイルス感染の発生に備え、平成17年10月25日付血液対策課発事務連絡に基づき緊急対応の準備を進めている。今後も引き続き情報の収集に努める。

(7)

JRC2008T-068

ABC Newsletter -9- October 17, 2008

WNV Case in Italy is First There in Many Years

Two human cases of West Nile Virus (WNV) encephalitis have been reported in Italy in the last month, the first human cases in that country in many years.

On September 20, the laboratory of the Regional Reference Center for Microbiological Emergencies in Bologna, Italy, reported the detection of specific IgM and IgG antibodies against WNV in the serum of a female patient in her 80s who lives in a rural area between Ferrara and Bologna.

Six confirmed cases of WNV disease in horses have recently been reported in this area, and 13 birds (six crows and seven magpies) have been identified as positive for WNV. Subsequently, an active surveillance program for possible human cases of WNV meningoencephalitis began.

Nucleic acid amplification testing has been introduced for blood donor screening in the provinces of Bologna and Ferrara. The Italian National Blood Center also has instructed all blood centers to defer for 28 days donors who have been for at least one night in the subject areas.

No Travel Reported. The patient had fever and repeat vomiting episodes on September 5. A first diagnosis of suspected urinary tract infection was made and the patient was given medication, but the symptoms remained and the patient was admitted to an Imola hospital on September 19 with high fever, vomiting, impaired consciousness, and hallucinations. The patient went into convulsions in the emergency room. She has regained consciousness and has almost completely recovered, though she remains hospitalized as a safety precaution.

Serum samples were tested for WNV-specific antibodies using an enzyme-linked immuno-sorbent assay, which indicated an acute WNV infection. WNV-specific antibodies were further confirmed by additional serological tests on the first samples. The samples were tested for Japanese encephalitis virus (JEV) and tick-borne encephalitis virus (TBEV). "Results clearly demonstrated that the antibody response was mainly directed against WNV, thus corroborating the hypothesis of a WNV neuroinvasive infection," according to the *Eurosurveillance Report* (10/9/08).

The patient's relatives reported that she had not traveled outside the small village where she has lived for the past two years. The patient's home is located within a few kilometers from a large swamp that is home to a sizeable population of different bird species and is infested by mosquitoes (both *Culex* and *Aedes albopictus*).

A second human case of WNV neuroinvasive disease was identified in Bologna on October 3 – a man in his late 60s who lived in the province of Ferrara where WNV-positive horses and birds have recently been identified. The patient suffered from symptoms of acute meningoencephalitis with high fever. Serum and cerebrospinal fluid samples of this patient have tested positive for IgG and IgM antibodies against WNV and two different RT-PCRs performed on the serum were positive, though confirmatory laboratory testing was still pending.

WNV has been reported in Europe, the Middle East, Africa, India, parts of Asia, and Australia. Human WNV disease has been reported in the Mediterranean Basin: in Algeria in 1994, Morocco in 1996, Tunisia in 1997 and 2003, Romania in 1996 through 2000, the Czech Republic in 1997, Israel in 1999 and 2000, Russia in 1999 through 2001, and France in 2003. Enzootics involving horses were reported in 2000, Morocco in 1996 and 2003, Italy in 1998, Israel in 2000, and southern France in 2000, 2003, and 2004. (Sources: *Eurosurveillance Report*, 10/9/08; European Commission response to European Blood Alliance query, 10/6/08) ♦

別紙様式第2-1

No. 22

医薬品 研究報告 調査報告書

識別番号・報告回数		報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
			2008. 11. 4	該当なし	
一般的名称	人全血液	研究報告の公表状況	Furtner M, Gelpi E, Kiechl S, Knoflach M, Zangerl A, Gotwald T, Willeit J, Maier H, Ströbel T, Unterberger U, Budka H. J Neurol Neurosurg Psychiatry. 2008 Feb;79(2):229-31.	公表国 オーストリア	
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

○ヒト成長ホルモンによる治療22年後に発症した医原性クロイツフェルト・ヤコブ病、臨床および放射線学的特徴
医原性のクロイツフェルト・ヤコブ病(iCJD)の多くは、プリオンに汚染されたヒト成長ホルモン(hGH)製剤の投与によるものである。
患者は、11歳でクッシング症候群と診断され、1984年9月から1985年11月まで死体から採取し市販用に製造されたhGH(クレスコモン、カビ社、現在は製造中止)の投与を受けていた。
2007年、神経学的兆候により入院後、状態は急速に悪化し、集中的な理学療法と言語療法にもかかわらず、患者は4ヵ月後に死亡した。
組織学的検査で海綿状の変化、神経細胞脱落、グリオーシスの特徴を示し、免疫組織学的検査は特異的なプリオン蛋白の沈着が見られた。医原性のリスクが認められたため、WHOの基準に従い確定iCJDに分類された。プリオン蛋白遺伝子(PRNP)には既知の突然変異は認められず、患者はPRNPコドン129、メチオニンホモ接合体であった。
疾患発症後の1、2、3ヵ月目に実施したMRIによる連続造影上の変化は、海綿状の変性を示しており、拡散強調画像の偽正常化は進行性の細胞死と関連していると推察された。
hGH投与22年後におけるCJD発症は、英国における一連のhGH-iCJD試験で推計された暴露後およそ20年というリスクのピークと一致する。
本症例は、hGHを投与された患者としては、オーストリアにおける初のCJD症例である。

使用上の注意記載状況・その他参考事項等

人全血液-LR「日赤」
照射人全血液-LR「日赤」

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

ヒト(死体)由来のヒト成長ホルモン(hGH)製剤の投与を受けた患者が、22年後にクロイツフェルト・ヤコブ病を発症し、4ヵ月後に死亡し、確定医原性CJDに分類されたとの報告である。
なお、日本においては1995年以降には、すべてリコンビナントヒト成長ホルモン製剤に切り替わった。

今後の対応

日本赤十字社では、CJDのリスクのある血液を排除する目的から、献血時にhGH製剤投与の有無を確認し、該当するドナーを無期限に献血延期としている。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

(10)

JRC2008T-065

PostScript

A novel mutation (c.64_65delGGinsAACC [p.G21fsX66]) in the *GTP cyclohydrolase 1* gene that causes Segawa disease

DYT5 dystonia (Segawa disease) is an autosomal-dominant inherited progressive dystonia that is evoked by mutations/deletions of the *GTP cyclohydrolase 1* (*GCH1*) gene,[1-3] which codes for the rate-limiting enzyme of tetrahydrobiopterin (BH_4) synthesis. Segawa disease is a rare disorder with an estimated prevalence of 0.5 per million. We report a clinical course caused by a novel mutation of the *GCH1* gene in a 25-year-old Caucasian female presenting in our outpatient clinic. The patient was born to healthy parents with no history or signs of neurological diseases. She described the development of a gait disturbance beginning at the age of 5 years. She was increasingly unable to walk at her soles, but was only walking at the outer edges of her feet (*pedes equinovarus*), causing a monstrous callus, within years. The feet cramped after only a few steps, which was relieved after some rest. Several stays in hospital did not reveal the final diagnosis, so that the gait disturbance was initially classified as a psychogenic disorder. The patient was then introduced to our movement disorder outpatient clinic just before an operation of the feet abnormalities. Clinical examination showed focal crampi of both feet with relevant relief only by inactivity. The feet were severely adducted and suppinated. Neurophysiological examinations, including somatosensory and magnetic-evoked potentials, were normal. A magnetic resonance imaging scan of the cervical and thoracic spine revealed only a short hydromyelia with no signs of inflammation or neoplasma. Analyses of the biogenic amines and pterins in the cerebrospinal fluid, according to the methods of Curtius and Hyland,[4,5] revealed highly decreased dopamine (homovanillic acid 48 nmol/l; normal values: 115–455) and serotonine metabolites (5-hydroxyindoleacetic acid 20 nmol/l; normal values: 51–204). Similarly, all pterines were markedly reduced (tetrahydrobiopterin: below detection level [normal value: 18–53 nmol/l]; total neopterin: 6 nmol/l [normal value: 10–31]). Folate metabolites were normal. To confirm the diagnosis of Segawa disease, GTP-cyclohydrolase I (GTPCH) enzyme activity was determined in skin fibroblasts according to Bonafé *et al.*,[6] which showed only 34% activity (0.99 μU/mg protein) compared with healthy controls (reference value: 2.6±0.53 μU/mg protein). Treatment with low doses of levodopa was capable of resolving the symptoms completely. Sequencing of exons 1–6 of the *GCH1* gene revealed a heterozygous deletion of two guanines at positions 64 and 65 and an insertion of 4 bases (AACC; fig 1), leading to a frameshift from amino acid 21 and subsequent termination of the protein after amino acid 66 within exon 1 (c.64_65delGGinsAACC [p.G21fsX66]). Multiplex ligation-dependent probe amplification (MRC, Amsterdam, The Netherlands) of the whole *GCH1* did not detect any further deletions. The clinically unaffected parents did not show any mutation in the *GCH1* gene (fig 1), confirming that the mutation in the patients represents a *de novo* mutation. This novel combined deletion-insertion mutation leading to protein truncation within exon 1 has not been reported before, despite up to more than 100 abnormalities of the *GCH1* gene being reported—including exon (start point change, missense, nonsense and frameshift mutations) and intron mutations, and deletions.

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Competing interests: None declared.

Received 25 July 2007
Revised 18 September 2007
Accepted 19 September 2007

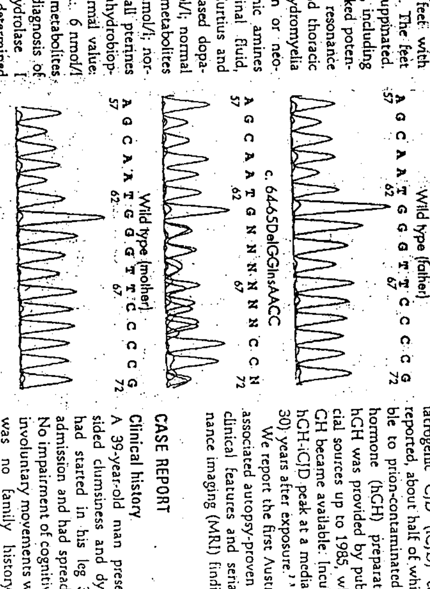

Figure 1 Genomic sequences of the index patient (middle panel) and both parents (father: upper panel; mother: lower panel), revealing a heterozygous deletion of two guanines at positions 64 and 65 and an insertion of the four bases AACC in the index patients, but wild-type sequences in both parents. The sequence abnormalities lead to a frame shift from amino acid 21 and subsequent termination of the protein after amino acid 66 within exon 1.

J Neurol Neurosurg Psychiatry 2008;79:229.
doi:10.1136/jnnp.2007.130849

REFERENCES

1. Segawa M, Nomura Y, Nishiyama N. Autosomal dominant guanosine triphosphate cyclohydrolase I deficiency (Segawa disease). *Ann Neurol* 2003;54(Suppl 6):S32–45.
2. Thony B, Blau N. Mutations in the BH4-metabolizing genes GTP cyclohydrolase I, 6-pyruvoyl-tetrahydropterin synthase, sepiapterin reductase, carbinolamine-4a-dehydratase, and dihydropteridine reductase. *Hum Mutat* 2006;27:870–8.
3. Hagenah J, Saunders-Pullman R, Hedrich K, *et al.* High mutation rate in dopa-responsive dystonia: detection with comprehensive GCHI screening. *Neurology* 2005;64:908–11.
4. Curtius HC, Blau N, Kuster T Pterins. In: Hommes FA, ed. Techniques in diagnostic human biochemical genetics New York: Wiley-Liss, 1991:377–96.
5. Hyland K, Surtees RA, Heales SJ, *et al.* Cerebrospinal fluid concentrations of pterins and metabolites of serotonin and dopamine in a pediatric reference population. *Pediatr Res* 1993;34:10–4.
6. Bonafé L, Thony B, Leimbacher W, *et al.* Diagnosis of dopa-responsive dystonia and other tetrahydrobiopterin disorders by the study of biopterin metabolism in fibroblasts. *Clin Chem* 2001;47:477–85.

Iatrogenic Creutzfeldt–Jakob disease 22 years after human growth hormone therapy: clinical and radiological features

Creutzfeldt–Jakob disease (CJD) is a human transmissible spongiform encephalopathy or prion disease. Although CJD is most frequently sporadic, numerous acquired or iatrogenic CJD (iCJD) cases have been reported, about half of which are attributable to prion-contaminated human growth hormone (hGH) preparations.[1] Cadaveric hGH was provided by public and commercial sources up to 1985, when recombinant GH became available. Incubation periods of hGH-iCJD peak at a median of 12 (range 5–30) years after exposure.[2,3]

We report the first Austrian case of hGH-associated autopsy-proven iCJD and discuss clinical features and serial magnetic resonance imaging (MRI) findings.

CASE REPORT

Clinical history

A 39-year-old man presented with right-sided clumsiness and dysaesthesia, which had started in his leg 3 weeks prior to admission and had spread to his right arm. No impairment of cognitive function and no involuntary movements were present. There was no family history of neurological disease. The patient had been healthy until the age of 11 years, when progressive obesity and growth impairment had been noticed and a diagnosis of Cushing syndrome had been made. The patient moved to Austria at the age of 15 years (1982) and was subsequently diagnosed with a hormone-producing pituitary adenoma, which was removed by transsphenoidal hypophysectomy. The frontal skull base defect was covered with

autologous connective tissue (fascia lata). Due to persistent Cushing syndrome symptoms, bilateral adrenalectomy was performed. To promote body growth (height <3rd percentile), he received commercially manufactured cadaveric hGH (Crescormon®, Kabi Pharma, now discontinued) from September 1984 (2 IU IM three times per week, which was later reduced to 2 IU IM twice a week). The treatment was continued until November 1985 and resulted in an increase of body height of 13.5 cm.

In 2003, a recurrency of the pituitary adenoma causing Cushing symptoms was diagnosed and transsphenoidal resection was performed, again with an autologous fascia lata graft.

On admission, the patient's neurological exam showed coarse bilateral gaze nystagmus, vertical gaze palsy and mild right-sided hemiparesis. Tendon reflexes in both lower extremities were exaggerated, whereas pyramidal signs were negative. Gait was paraspastic, with a deviation tendency to the right, but unaided walking was still possible. Cerebellar tests revealed bilateral ataxia in the upper and lower limbs and dysdiadochokinesia of both hands. Testing for infectious, parainfectious, as well as neoplastic or paraneoplastic neurological diseases, was negative, as was metabolic screening.

Serial cerebral MRI was performed in months 1, 2 and 3 (fig 1). Electroencephalographic recordings (EEGs) in months 1 and 2 showed diffuse slowing with generalized delta activity and intermittent rhythmic delta-theta runs with a right fronto-central accentuation. EEG in month 3 revealed further slowing and some non-periodic bilateral sharp/slow wave complexes.

Cerebrospinal fluid (CSF) examinations in week 1 and week 6 after admission exhibited divergent results. In the first sample, 14-3-3 protein was undetectable; protein content, as well as cytology, were normal. In the second CSF sample, a strong signal in the molecular weight range of the 14-3-3 protein was detected.

Neuropsychological examination 3 weeks after admission showed reduction of attentive functions, whereas memory was unimpaired. Over 3 months of hospitalization, the patients condition rapidly deteriorated. Myoclonus of both arms and legs emerged; the patient became bedridden after about 6 weeks. Speech was increasingly dysarthric, and severe dysphagia ensued. Hypostatic pneumonia required antibiotic treatment. Despite intensive physiotherapy and speech therapy, the patient's condition continued to worsen. The patient died after an overall disease course of 4 months.

Neuropathology

Histology showed the characteristic triad of spongiform change, neuronal loss and gliosis. Immunohistochemistry revealed characteristic prion protein deposits in cerebral and cerebellar cortices, confirming the diagnosis of CJD. Due to the recognised iatrogenic risk (hGH), the disease was classified as definite iCJD according to World Health Organization (WHO) criteria.[1] Western-blot analysis of proteinase K restistant PrP was not performed due to lack of adequate material.

Genetic analysis

Sequencing of the entire coding region of the prion protein gene (*PRNP*) performed after isolation of genomic DNA from peripheral blood showed no known mutations. The patient was methionine homozygous at codon 129 of the *PRNP*.

DISCUSSION

This case of definite iatrogenic CJD 22 years after hGH medication exhibits several noteworthy features.

MRI studies 1, 2 and 3 months after manifestation of disease revealed early bilateral cortical involvement of the mesial frontal lobes. Diffusion-weighted imaging (DWI) hyperintensities progressed to adjacent cortical areas and to the striatum, in line with clinical deterioration (fig 1). DWI has been recommended as the most sensitive test for early diagnosis of CJD,[2] but is not suggestive of a specific form of disease. HGH-iCJD cases have exhibited DWI hyperintensities mainly in the basal ganglia. Cerebellar malfunction is one of the most common early signs of iCJD after hGH treatment[1] and was one of the main clinical disturbances at disease onset in our patient. However, no corresponding MRI abnormalities were detected in the cerebellum. To our knowledge, no other hGH-iCJD case has been documented with early frontomesial DWI changes and progressive bilateral striate hyperintensities.

CSF 14-3-3 protein was negative on first testing and turned positive 4 weeks later. Of interest, DWI changes preceded CSF 14-3-3 protein conversion by weeks and had spread from the cortical distribution shown in figure 1A/B to a striatal DWI pattern that is commonly associated with sporadic CJD (fig 1B). It has been speculated that these changes on serial imaging indicate spongiform degeneration, but that the neurons are still viable in the early disease stages, and that a subsequent DWI pseudonormalization is related to progressive cell death.[4]

The clinical presentation, with paraspastic gait as one of the first striking features, also requires attention. This correlates well with the imaging findings and represents a bilateral parietal edge syndrome—that is, first motoneuron dysfunction in the leg areas of both precentral gyri.

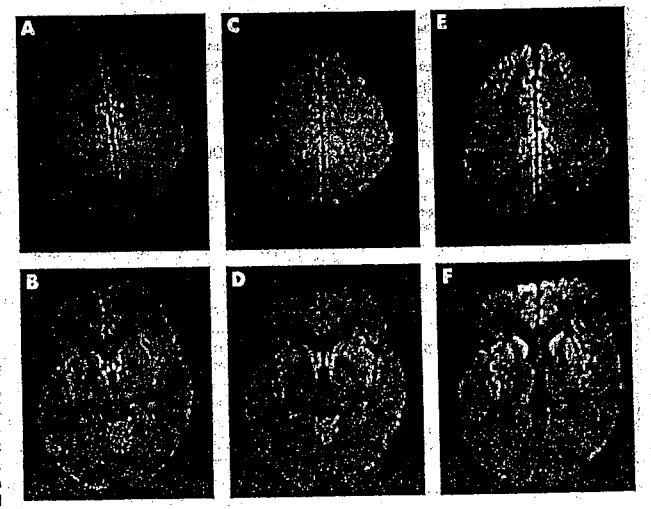

Figure 1 Magnetic resonance imaging (MRI) 1 month (panels A and B), 2 months (C, D) and 3 months (E, F) after onset. Diffusion weighted imaging (DWI) 1 month after onset revealed bilateral frontomesial hyperintensities (A), and moderate DWI signal increases in the medial portion of both caudate heads (B). Two months after onset, the bifrontal hyperintensities showed slight enlargement (C), and DWI signals were elevated in both caudate heads, the adjacent putamina and insular cortices (D). On follow-up MRI 1 month later, there was increased DWI signal in the frontomesial and frontopolar cortex (E,F) and marked DWI hyperintensity in both caudate heads, both putamina with accentuation in their rostral parts, and both insular ribbons (F). ADC maps and FLAIR images were inconspicuous (data not shown).

Occurrence of CJD 22 years after hGH administration is in line with the peak risk approximately 20 years after exposure calculated from a large hCH-iCJD series in the UK,[3] whereas the mean incubation period in French hGH recipients was considerably shorter at 9–10 years.[5] Differences of infectivity in hormone lots have been suggested as an explanation for this finding.

Some unusual circumstances and clinical features also deserve comment. First, iCJD associated with hGH has, so far, only been reported after administration of non-commercial hormone. The reports available, however, have excluded patients treated with commercially prepared hormone; hence, there are insufficient data on the CJD rate in these patients.[1 5] Second, the administration period of hGH and disease duration were both short for iCJD patients even though comparable cases have been reported in previous literature.[2 7]

In summary, this is the first CJD case from Austria in a patient having received hGH and only the third iatrogenic case detected in this country. The recognised iatrogenic risk (cadaveric hGH 22 years before onset) and the neuropathological confirmation of CJD meet the WHO criteria for definite iCJD, although the possibility of a sporadic methionine-homocygous juvenile CJD case without causal relation to hGH treatment cannot be definitely ruled out.

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Competing interests: None.

Additional data are published online only at http://jnnp.bmj.com/content/vol79/issue2

Accepted 20 September 2007

J Neurol Neurosurg Psychiatry 2008;79:229–231. doi:10.1136/jnnp.2007.122267

REFERENCES

1. **Brown P**, Preece M, Brandel JP, *et al.* Iatrogenic Creutzfeldt-Jakob disease at the millennium. *Neurology* 2000;55:1075–81.
2. **Swerdlow AJ**, Higgins CD, Adlard P, *et al.* Creutzfeldt-Jakob disease in United Kingdom patients treated with human pituitary growth hormone. *Neurology* 2003;61:783–91.
3. **Huillard d'Aignaux J**, Costagliola D, Maccario J, *et al.* Incubation period of Creutzfeldt-Jakob disease in human growth hormone recipients in France. *Neurology* 1999;53:1197–201.
4. **World Health Organisation.** WHO manual for surveillance of human transmissible spongiform encephalopathies including variant Creutzfeldt-Jakob disease. *WHO Communicable Disease Surveillance and Response* 2003.
5. **Shiga Y**, Miyazawa K, Sato S, *et al.* Diffusion-weighted MRI abnormalities as an early diagnostic marker for Creutzfeldt-Jakob disease. *Neurology* 2004;63:443–9.
6. **Oppenheim C**, Zuber M, Galanaud D, *et al.* Spectroscopy and serial diffusion MR findings in hGH-Creutzfeldt-Jakob disease. *J Neurol Neurosurg Psychiatry* 2004;75:1066–9.
7. **Croes EA**, Roks G, Jansen GH, *et al.* Creutzfeldt-Jakob disease 38 years after diagnostic use of human growth hormone. *J Neurol Neurosurg Psychiatry* 2002;72:792–3.

APPENDIX

Histopathological examination

The total fixed brain weight was 1408 g. Macroscopically, moderate diffuse cerebral and cerebellar atrophy was observed. In addition, there were signs of diffuse oedema. On coronal sections, the cortical ribbon of the insular and parietal cortices was narrowed. Histology showed characteristic spongiform change, moderate neuronal loss and gliosis in cerebral cortex and basal ganglia (see Supplementary figure). The cerebellar cortex was severely affected with marked spongiform change of the molecular layer and neuronal loss of the granule cell layer (see Supplementary figure). The Purkinje cells and brain stem nuclei were comparatively better preserved. Immunohistochemistry using the antibody 12F10 (Cayman, Ann Arbor, Michigan, USA) revealed strong pathological prion protein (PrP^{sc}) deposits in cerebral and cerebellar cortices, and basal ganglia in a diffuse synaptic pattern (see Supplementary figure). In the brain stem nuclei, only discrete PrP^{sc} deposits were demonstrable. There were no PrP^{sc} plaques neither in the cerebellum nor in the cerebral cortex or white matter. These features confirmed the diagnosis of Creutzfeldt–Jakob disease (CJD). Due to the recognised iatrogenic risk (due to human growth hormone), the disease was classified as definite iatrogenically transmitted CJD, according to World Health Organisation criteria.

Skin reactions after intramuscular injection of Botulinum toxin A: a rare side effect

The use of Botulinum toxin (BTX) has been constantly increasing over the past years, not least on account of obtaining the license for the treatment of facial lines. It has proven a safe drug with only a few adverse effects. Local irritations at the injection site are not uncommon, whereas more widespread and generalised exanthemas were first described in 1992.[1] One dramatic case documents a lethal outcome due to treatment with a mixture of BOTOX® (BTX-A) and lidocaine.[2] In accordance with databases from the companies Allergan and Ipsen (SPC BOTOX®, Allergan, December 2005; SPC DYSPORT®, Ipsen Pharma, April 2006), skin reactions seem to be a rare phenomenon with a frequency of less than 1:1,000. The Ipsen database (January 2007) mentions 5 cases of local and 4 cases of more widespread redness, bulging and pruritus in Germany, as well as 11 cases abroad. Here, we report on two further cases of rapid-onset skin reactions after injection of two different BTX-A products.

CASE 1

A 49-year-old woman developed a left-sided spastic hemiparesis after cavernoma exstirpation in 1997. Successful treatment of the spastic arm muscles was carried out with BOTOX® for about 5 years and with DYSPORT® for the last 4 years. She did not receive any other medication. Injection intervals ranged from 3 to 9 months. During the treatment session in April 2006, we applied a total dose of 1,000 Units DYSPORT® (250 MU into the left biceps muscle, 250 MU into the left flexor pollicis longus and extensor carpi radialis muscles, 500 MU into the left flexor digitorum superficialis muscle). Within 6 hours after intramuscular injection of BTX-A, a segmental or "pseudosegmental" fine-spotted pruriginous exanthema emerged in the region of the entire left shoulder, arm and left breast. Fever or other additional symptoms did not occur. Allergological tests, such as prick tests, and an intracutaneous test were normal. Treatment with DYSPORT® was repeated 3 months later with a dose reduction of 50% without any adverse effects. At a later visit, she received 1,000 Units DYSPORT®, which was well tolerated.

CASE 2

A 63-year-old man presented with right-sided limb spasticity due to a stroke 7 years ago. The patient received a stable medication consisting of gabapentine, tramadole, tetrazepam, clopidogrel and atorvastatin. From 2003, he was successfully treated with injections of 900–1,100 Units DYSPORT® at regular intervals of 3 months. In 2006, the therapy was changed to BOTOX®. Within

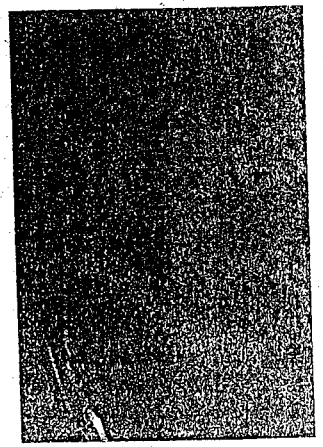

Figure 1 Photograph of the skin reaction as described in Case 2 about 1 hour after injection into the right brachial muscle. Informed consent was obtained for publication of this figure.

別紙様式第２－１

医薬品　研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
一般的名称	－		研究報告の公表状況	Laboratory Hematology (United States) 2007, 13 (1) p34-8	公表国 米国	
販売名（企業名）	－					

研究報告の概要

重症筋無力症の治療として行ったアルブミンを交換液とした血漿交換の後に、パルボウイルス B19（以下「B19」）感染による赤芽球癆を発症した女性の症例を報告する。
アルブミン投与から 2 週間後に、患者は網状赤血球欠乏性貧血を発症し、骨髄穿刺を行ったところ、多数の巨大な前正赤芽球欠乏を伴う顕著な一連の低形成赤血球が示され、重度網状赤血球減少症を伴う貧血および骨髄の形態によって、B19 感染が原因の赤芽球癆が疑われ、IgM および IgG 型抗 B19 抗体により確認された。
患者は免疫グロブリン（0.4g/kg、4 日間）で治療したところ、貧血は徐々に回復した。
アルブミン、凝固因子、免疫グロブリンなどの血液製剤の感染性は除外できず、血液成分による B19 感染は依然未解明の問題である。
B19 はエンベロープを有さないウイルスであるため、溶媒－界面活性剤処理には抵抗性であるが、60℃で 10 時間低温殺菌すると迅速に不活化することを示したとの報告もある。
ウイルス不活化の新たな方法や B19 陽性単位の棄却などの多くの戦略は、血液製剤の安全性を増すのに有用である。

使用上の注意記載状況・その他参考事項等

慎重投与（次の患者には慎重に投与すること）
・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。］
・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。］
重要な基本的注意
(1) 本剤の原材料となる‥［スクリーニング項目、不活化・除去工程］‥投与に際しては、次の点に十分注意すること。
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。
妊婦、産婦、授乳婦等への投与
妊婦又は妊娠している可能性のある婦人には治療上の有益性が危険性を上回ると判断される場合にのみ投与すること。［妊娠中の投与に関する安全性は確立していない。本剤の投与によりヒトパルボウイルス B19 の感染の可能性を否定できない。感染した場合には胎児への障害（流産、胎児水腫、胎児死亡）が起こる可能性がある。］

報告企業の意見	今後の対応
アルブミン投与後にパルボウイルス B19 感染が疑われた症例の報告である。 当社血漿分画製剤は最終製品において NAT 検査を行い、パルボウイルス B19DNA 陰性であることを確認している。	今後ともパルボウイルス B19 に関する血漿分画製剤の安全性に関する情報に留意していく。

NIHONSEIYAKU
2008-008

Laboratory Hematology 13:34-38
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doi: 10.1532/LH96.06036

CASE REPORT

Parvovirus B19 Infection after Plasma Exchange for Myasthenia Gravis

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Received September 11, 2006; accepted October 24, 2006

ABSTRACT

We describe a case of pure red cell aplasia caused by a B19 parvovirus infection in a female myasthenic patient treated with plasma exchange, corticosteroids, and cholinesterase inhibitors. Two weeks after albumin infusion, she developed anemia with an absence of reticulocytes. A bone marrow aspirate was performed, showing a markedly hypoplastic erythroid series with numerous giant pronormoblasts. Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive immunoglobulin (Ig)M and IgG anti-B19 virus. The patient successfully responded to IVIG treatment with a complete remission. In this case, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Although human B19 DNA content does not reflect infectivity, it is not possible to exclude that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. Actually, blood component B19 infection is still an unresolved problem. Many strategies such as new methods for viral inactivation and discarding positive B19 units may help to increase blood product safety. *Lab Hematol* 2007;13:34-38.

KEYWORDS: Parvovirus B19 • Pure red cell aplasia • Albumin • Myasthenia gravis • Plasma exchange

INTRODUCTION

Parvovirus B19 is a single-stranded DNA virus, forming small capsides and lacking a lipid envelope. Its genome encodes 3 major viral proteins, VP1 and VP2, the viral capsid proteins, which lead to self-assembly of viral particles, and NS1, a nonstructural protein, which is responsible for cytotoxicity. It has a peculiar tropism for human erythroid progenitors, with inhibition of erythroid colony growth and cytopathic effect [1-2].

B19 parvovirus is a common infection in humans, and about 50% of adults have immunoglobulin (Ig)G antibodies against the virus. Parvovirus infection is common in childhood and continues at a low rate throughout adult life. Most cases of parvovirus infection are asymptomatic. The most common clinical presentation is fifth disease of childhood, characterized by typical exanthema, fever, and flu-like symptoms. Acute or chronic arthropathy due to deposition of immune complexes may occur in adults. In patients with chronic hemolytic anemia, such as hereditary spherocytosis and sickle cell disease, acute parvovirus B19 infection can cause an abrupt cessation of red cell production, with transient aplastic crisis. In patients with immunodeficiency states, such as congenital immunodeficiencies or AIDS and patients receiving cytotoxic chemotherapy or immunosuppressive drugs, such as administered after an organ transplantation, there can be a failure to produce neutralizing antibodies. In these cases, pure red cell apla-

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sia can develop, with an absence of circulating reticulocytes and giant pronormoblasts in the bone marrow, without maturing normoblasts. Hydrops fetalis from transplacental infection and usually transitory hemophagocytic syndrome are other clinical disorders caused by B19 [3].

Parvovirus B19 transmission by blood products and plasma derivates, such as albumin, clotting factor concentrates, and intravenous immunoglobulin (IVIG) has been repeatedly demonstrated [4]. Transmissibility in coagulation products has occurred among patients who received heat-treated, pasteurized, monoclonally purified and solvent-detergent–treated concentrates [5]. Infection with B19 due to transfusion with cellular blood products is a rare event, but it has been reported twice with red blood cells and once with platelets [6-8]. We report a case of a myasthenic patient with pure red cell aplasia due to a parvovirus B19 infection.

CLINICAL CASE DESCRIPTION

In 1997, a 29-year-old woman complained of intermittent speaking difficulty (dysarthria). In April 1998, 10 days before the full-term delivery of her second healthy baby, more severe symptoms appeared, such as facial nerve and oro-pharyngeal deficit and weakness of the arms and legs. Ten days after delivery, the patient was admitted to a hospital for a typical myasthenic crisis with severe weakening of respiratory muscles, requiring a respirator to assist ventilation. Treatment was started with 4 consecutive plasma exchanges and administration of corticosteroids and cholinesterase inhibitors (pyridostigmine bromide) with marked clinical improvement. In August 1998, the patient withdrew from medical therapy, which led to a worsening of symptoms and a new hospitalization in a different institution. There she was treated with 5 therapeutic plasma exchanges using albumin as replacement fluid. Medical treatment was started again. On August 31, she had a deep vein thrombosis, treated with IV heparin. On September 3, she was admitted to the Neurology Department of our hospital. At admission, the patient had normochromic-normocytic anemia (hemoglobin [Hgb], 97 g/L), with normal platelet and white blood cell counts.

Two weeks later, anemia worsened and was associated with thrombocytopenia (Hgb, 81 g/L; platelets, 57×10^9/L) (Figure 1). Schistocytes were absent. A diagnosis of heparin-induced thrombocytopenia was made. Heparin tapering was started, and the platelet count improved. A few days later, since anemia was still severe (Hgb, 80 g/L) and of an aregenerative type with an absence of reticulocytes, a bone marrow aspirate was performed. This showed many moderate hypercellular marrow particles and an increased number of megakaryocytes. An erythroid series was markedly hypoplastic with complete maturative arrest. The only visible erythroid precursors were giant pronormoblasts with vacuolated deep basophilic cytoplasm, sometimes grouped in clusters simulating metastatic cells (Figures 2 and 3). Anemia with severe reticulocytopenia and morphology of bone marrow suggested a diagnosis of pure erythroblastopenia due to parvovirus B19 infection, which was confirmed by positive tests for IgM and IgG anti-B19 virus. Increased megakaryocytes tended to confirm that thrombocytopenia was heparin-induced. The patient was treated with immune globulin (0.4 g/kg for 4 days). Reticulocytosis appeared on September 30 (202×10^9/L; normal values, 30-90 $\times 10^9$/L). Anemia recovered slowly (Hgb, 92 g/L at discharge), and thrombocytopenia completely regressed. The patient was admitted again to

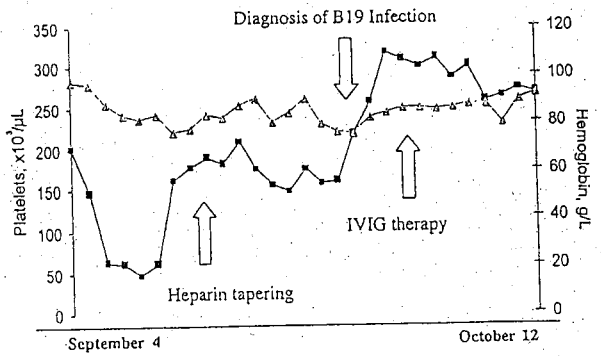

FIGURE 1. Hematological values and clinical course of the patient from admission (September 4, 1998) to discharge (October, 12 1998). Triangle indicates platelet count; square, hemoglobin concentration.

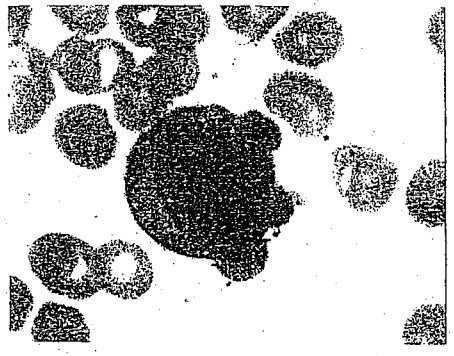

FIGURE 2. Basophilic giant pronormoblast with pseudopodia or "dog ears."

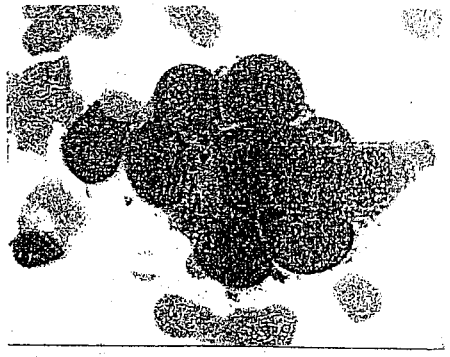

FIGURE 3. A cluster of pronormoblasts with maturative arrest.

the hospital in May 1999 for surgical resection of a thymoma. At that time, her full blood count was normal, IgM anti-B19 was negative, and IgG anti-B19 was still positive.

DISCUSSION

We described a case of pure red cell aplasia caused by parvovirus B19 in a patient with myasthenia gravis treated with plasma exchanges using albumin, corticosteroids, and cholinesterase inhibitors.

Parvovirus B19 has a particular tropism for erythroid progenitors. The cellular receptor for B19 is erythrocyte P antigen, a globoside that consists of a long-chain fatty acid on a ceramide back-bone structure with 4 sugar residues ending with terminal N-acetyl galactosamine. The P antigen is a common erythrocyte and erythroblast antigen, and it is expressed in almost all subjects. People who lack the P antigen are resistant to infection [1]. In this case, the patient had P_1 phenotype, which is the most common phenotype among Caucasians (79%) and Africans (94%). P_2 phenotype is more common among Asian people, such as Cambodians and Vietnamese. [9].

P antigen is also expressed on megakaryocytes, endothelial cells, synovium, villous trophoblast cells of placental tissues, fetal liver, and heart cells. B19 infection may also be responsible for thrombocytopenia, and megakaryocytes may be lysed by restricted expression of viral proteins in the absence of viral propagation [10]. In this case, thrombocytopenia was heparin-induced, confirmed by an increase of the peripheral platelet count when heparin tapering was started (Figure 1). Heparin-induced thrombocytopenia is more often reported after orthopedic, cardiac, or vascular surgery, but it may develop in any patient exposed to unfractionated heparin or low molecular weight heparin [11]. Furthermore, the patient's bone marrow showed increased megakaryocytes, which tended to confirm that thrombocytopenia was heparin induced.

After binding with P antigen, the virus enters the targeted cells, probably because of the VP1 phospholipase activity, and starts to synthesize viral components. It has been demonstrated that B19 is a potent inhibitor of erythroid cell differentiation, and it is cytotoxic for erythroid precursors. It acts by inducing apoptosis through the activation of the caspase pathway or direct lytic effect on erythroid cells. Apoptosis is mediated by NS1 expression, which induces activation of caspase-3, caspase-6, and caspase-8 in a cellular model [12,13].

The virus is also responsible for a cytopathic effect on cells causing a maturative arrest in the erythroid cell line. In smears from bone marrow aspirate, the pathognomonic cell for B19 infection is the giant proerythroblast, which is a large cell, from 25 to 32 µm in diameter, with a high nucleo-cytoplasmic ratio; the nucleus is round and it has a fine and uncondensed chromatin pattern with irregular, indistinct purple-colored inclusions. A giant proerythroblast has a dark blue vacuolated cytoplasm with small broad-based cytoplasmic pseudopodia, named "dog-ear" projections. Sometimes they are grouped in clusters simulating metastatic cells [14]. As shown in Figures 2 and 3, the patient's bone marrow was characterized by the presence of large numbers of these immature erythroid cells. This accounts for anemia with severe reticulocytopenia, sometimes requiring red blood cell transfusions.

In patients with chronic hemolytic disorders, such as sickle cell disease and spherocytosis, B19 may cause transient aplastic crisis characterized by aregenerative acute anemia, sometimes associated with pancytopenia. Persisting B19 infection can occur in a wide variety of conditions, including congenital immunodeficiencies, HIV infection, lymphoproliferative disorders, and transplantation. In these cases, patients may have chronic pure red cell aplasia and more

rarely pancytopenia [15]. In pregnant women, parvovirus B19 may be transmitted to the fetus and may lead to miscarriage or hydrops fetalis [16].

Although the presence of giant proerythroblasts is suggestive of B19 infection, the diagnosis should be made by serological detection of antibodies or molecular detection of viral components. Serological determination of antibodies may be performed by enzyme-linked immunosorbent assays that are able to identify IgM and IgG antibodies. IgM antibodies remain detectable for 2 or 3 months following the infection, as opposed to IgG antibodies which appear 2 weeks after the infection but persist for life. Immunocompromised patients sometimes are not able to produce IgM, and in these cases molecular tests, such as direct hybridization and gene-amplification methods, may be helpful to confirm a clinical suspicion [2].For our patient, tests gave positive results for IgG and IgM at the time of the diagnosis. Some months later, because of a further admission, her test results for IgM anti-B19 were negative, while those for IgG anti-B19 were still positive. At that time, molecular tests were not performed.

In children and immunocompetent adults, B19 infection does not require any treatment. In patients with immunodeficiencies or pure red cell aplasia, treatment with IVIG may be helpful and should be associated with discontinuing immunosuppressive drugs. Generally a 5- or 10-day course of IVIG (0.4 g/kg of body weight) causes a rapid virus elimination associated with reticulocytosis and elevation of Hgb concentration [17].

B19 may be transmitted by respiratory droplets, but secondary infection among households and nosocomial infection have been described [18,19]. B19 transmission by blood products and derivates, such as IVIG [20], solvent-detergent–treated pooled plasma [21], and clotting factor concentrates [5] has been repeatedly demonstrated, even after viral inactivation methods.

B19 is an envelope-free virus and therefore resistant to solvent-detergent treatment. This treatment is effective for clearance of HBV, HCV, and HIV, but it is not effective for HAV and B19, both of which lack the envelope. B19 resistance to heat is controversial. The virus is relatively heat stable [21], but Blümel et al [22] showed that pasteurization for 10 hours at 60°C rapidly inactivates B19. Although human B19 DNA content does not reflect infectivity, we cannot exclude the possibility that blood derivates, such as albumin, clot factors, and immune globulin may be infectious. In our patient, we could not confirm whether an albumin-derived infection combined with a concomitant immunocompromised condition due to myasthenia and immunosuppressive treatment was responsible for the disease. Blood component B19 infection is still an unresolved problem. Many strategies such as new methods for viral inactivation and discarding positive-B19 units [23-25] may help to increase blood product safety.

REFERENCES

1. Chisaka H, Morita E, Yaegashi N, Sugamura K. Parvovirus B19 and the pathogenesis of anaemia. *Rev Med Virol.* 2003;13:347-359.
2. Heegaard ED, Brown KE. Human parvovirus B19. *Clin Microbiol Rev.* 2002;15:485-505.
3. Young NS, Brown KE. Parvovirus B19. *N Engl J Med.* 2004;350:586-597.
4. Schmidt I, Blümel J, Seitz H, Willkommen H, Löwer J. Parvovirus B19 DNA in plasma pools and plasma derivatives. *Vox Sang.* 2001;81:228-235.
5. Yee TT, Cohen BJ, Pasi KS, et al. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. *Br J Haematol.* 1996;93:457-459.
6. Jordan J, Tiangco B, Kiss J, et al. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. *Vox Sang.* 1998;75:97-102.
7. Cohen BJ, Beard S, Knowles WA, et al. Chronic anemia due to parvovirus B19 infection in a bone marrow transplant patient after platelet transfusion. *Transfusion.* 1997;37:947-952.
8. Zanella A, Rossi F, Cesana C, et al. Transfusion-transmitted human parvovirus B19 infection in a thalassemic patient. *Transfusion.* 1995;35:769-772.
9. Reid ME, Lomas-Francis C. *The Blood Group Antigen FactsBook.* 2nd ed. New York, NY: Academic Press; 2004.
10. Srivastava A, Bruno E, Briddell R, et al. Parvovirus B19-induced perturbation of human megacaryocytopoiesis in vitro. *Blood.* 1990;76:1997-2004.
11. Bartholomew JR. The incidence and clinical features of heparin-induced thrombocytopenia. *Semin Hematol.* 2005;42:S3-S8.
12. Moffat S, Yaegashi N, Tada K, et al. Human parvovirus B19 nonstructural (NS1) protein induces apoptosis in erythroid lineage cells. *J Virol.* 1998;74:3018-3028.
13. Sol N, Le Junter J, Vassias I, et al. Possible interactions between the NS-1 protein and tumor necrosis factor alpha pathways in erythroid cell apoptosis induced by human parvovirus B19. *J Virol.* 1999;73:8762-8770.
14. Koduri PR. Novel cytomorphology of the giant proerythroblasts of parvovirus B19 infection. *Am J Hematol.* 1998;58:95-99.
15. Fisch P, Handgretinger R, Schaefer HS. Pure red cell aplasia. *Br J Haematol.* 2000;111:1010-1022.
16. Xu J, Raff TC, Muallem NS, Neubert AG. Hydrops fetalis secondary to parvovirus B19 infections. *J Am Board Fam Pract.* 2003;16:63-68.
17. Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19-mediated pure red-cell aplasia. *Autoimmun Rev.* 2005;4:264-269.
18. Chorba TL, Coccia P, Holman RC, et al. The role of parvovirus B19 in aplastic crisis and erythema infectiosum (fifth disease). *J Infect Dis.* 1986;154:383-393.
19. Bell LM, Naides SJ, Stoffman P, Hodinka RL, Plotkin SA. Human parvovirus B19 infection among hospital staff members after contact with infected patients. *N Engl J Med.* 1989;321:485-491.
20. Hayakawa F, Imada K, Towatari M, Saito H. Life-threatening

human parvovirus B19 infection transmitted by intravenous immune globulin. *Br J Haematol.* 2002;118:1187-1189.
21. Koenigbauer UF, Eastlund T, Day JW. Clinical illness due to parvovirus B19 infection after infusion of solvent detergent-treated pooled plasma. *Transfusion.* 2000;40:1203-1206.
22. Blümel J, Schmidt I, Willkommen H, Löwer J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. *Transfusion.* 2002;42:1011-1018.
23. Gallinella G, Moretti E, Nardi G, et al. Analysis of B19 Virus contamination in plasma pools for manufacturing by using a competitive polymerase chain reaction assays. *Vox Sang.* 2002;83:324-331.
24. Hitzler WE, Runkel S. Prevalence of human parvovirus B19 in blood donors as determined by a haemagglutination assay and verified by the polymerase chain reaction. *Vox Sang.* 2002;82:18-23.
25. Aubin JT, Defer C, Vidaud M, Maniez Montreuil M, Flan B. Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. *Vox Sang.* 2000;78:7-12.

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No. 13

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 9. 18	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	Kantele A, Marti H, Felger I, Müller D, Jokiranta TS. Emerg Infect Dis. 2008 Sep;14(9):1434-6.	公表国 フィンランド	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要

○マレーシアから帰国したヨーロッパ人旅行者におけるサルマラリア

2007年にマレー半島でフィンランドの旅行者が*Plasmodium knowlesi*に感染した。

患者は53歳男性で、マレー半島を4週間旅行してフィンランドに帰国した3日後に高熱を発症し、翌日受診した。患者ははじめの2週間クアラルンプールに滞在し、周辺地域を数日間旅行した。その後自動車で北西の海岸部に向かい5日間イポー近くのジャングルで過ごした。この間蚊帳のない家に泊まり防虫剤は使用していなかったが、蚊に刺されたという報告はなかった。最後の週はランカウイ・ビーチの高級ホテルに滞在していた。

血液塗抹検査でマラリア原虫が陽性となり、入院後塩酸キニーネとドキシサイクリンを合計10日間投与された。回復後12ヶ月間のフォローアップ期間中に再発は見られなかった。PCR産生物のヌクレオチド配列解析を行ったところGenBankに登録されていた*P. knowlesi*と一致した。

*P. knowlesi*は通常サルにマラリアを引き起こす寄生虫であるが、ヒトマラリアを引き起こす可能性がある第5のマラリア原虫(*Plasmodium* species)とされている。当該疾患はヒトの生命を脅かす恐れがあり、臨床医や臨床検査技師は、旅行者の当該病原体についての認識を高めるべきである。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見	今後の対応
2007年にマレー半島でフィンランドの旅行者が、通常サルにマラリアを引き起こす*Plasmodium knowlesi*に感染し、帰国後に発症したとの報告である。	日本赤十字社では、輸血感染症対策として問診時に海外渡航歴の有無を確認し、帰国(入国)後4週間は献血不適としている。また、マラリア流行地への旅行者または居住経験者の献血を一定期間延期している(1～3年の延期を行うとともに、帰国(入国)後マラリアを思わせる症状があった場合は、感染が否定されるまでの間についても献血を見合わせる)。今後も引き続き、マラリア感染に関する新たな知見及び情報の収集、対応に努める。

(2)

DISPATCHES

Monkey Malaria in a European Traveler Returning from Malaysia

Anu Kantele, Hanspeter Marti, Ingrid Felger, Danila Müller, and T. Sakari Jokiranta

In 2007, a Finnish traveler was infected in Peninsular Malaysia with *Plasmodium knowlesi*, a parasite that usually causes malaria in monkeys. *P. knowlesi* has established itself as the fifth *Plasmodium* species that can cause human malaria. The disease is potentially life-threatening in humans; clinicians and laboratory personnel should become more aware of this pathogen in travelers.

Traditionally, only 4 *Plasmodium* species have been known to cause malaria in humans: *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae*, although >26 *Plasmodium* species are known to circulate among primate populations (*1*). Some of these species have been implicated in symptomatic human malaria after experimental or accidental infection (*2*). Only a few reports of naturally acquired monkey malaria in humans are currently available (*1*,*3*–*9*). The lack of data may be because light microscopy has been used as the sole diagnostic method and an atypical *Plasmodium* species may have been misidentified as one of the 4 traditional *Plasmodium* species causing human malaria.

P. knowlesi was first described in 1931 in a long-tailed macaque imported from Singapore to India; in 1932, *P. knowlesi* was experimentally shown to be infectious to humans (*10*). The first natural infection of *P. knowlesi* in humans was reported in 1965 in a man returning to the United States after a visit to Peninsular Malaysia (*11*). Subsequently, in 1971, there was a report of a presumed natural infection in a citizen of Malaysia (*6*). Despite extensive studies in Malaysia in the 1960s (*2*), no other reports were published on naturally acquired *P. knowlesi* infections in humans until 2004, when Singh et al. studied PCR-negative *P. malariae* cases in the Kapit division in Sarawak, Malaysia (*3*). A different PCR analysis showed that *P. knowlesi* caused 58% of the 208 malaria cases studied. Further cases reported from China (*4*), Thailand (*5*), Philippines (*8*), and Singapore (*12*) show that *P. knowlesi* infections in humans are not found exclusively in Malaysia. Recently, Cox-Singh et al. reported that *P. knowlesi* is widely distributed among inhabitants of Malaysia (*7*).

The Study

A 53-year-old Finnish man was admitted to a local hospital in Finland in March 2007 with fever after 4 weeks of travel in Peninsular Malaysia. He had not taken any antimalarial prophylaxis. In Malaysia, he spent 2 weeks in Kuala Lumpur and made a few day trips to surrounding rural areas. Thereafter, he traveled by car to the northwestern coast and stayed for 5 days in the jungle ≈80 km south of Ipoh. While in this area, he slept in a house without mosquito screens or nets and did not use any repellents; he did not report any mosquito bites. The last week of his travel was spent in the Langkawi Beach area where he stayed at a high-quality hotel. During his trip he occasionally had some minor abdominal problems, but these symptoms subsided spontaneously after his return to Finland. High fever (38.8°C axillary temperature) occurred 3 days after his return to Finland but abated quickly. On the fourth day, the fever returned and he sought medical care at a local hospital. Laboratory tests showed the following results: C-reactive protein 2.0 mg/dL (normal range <1.0 mg/dL), hemoglobin 15.2 g/dL (normal range 13.4–16.7 g/dL), leukocyte count 2.6 × 10⁹/L (normal range 3.4–8.2 × 10⁹/L), and thrombocytes 143 × 10⁹/L (normal range 150–360 × 10⁹/L). Blood smear was positive for *Plasmodium* organisms, and the causative agent was identified as *P. falciparum* with levels of parasitemia <1.0%. The patient was admitted to the hospital and given intravenous (IV) quinine dihydrochloride and oral doxycycline.

On day 2 of the patient's hospital stay, fever returned and he was transferred to the Helsinki University Central Hospital (Department of Infectious Diseases at Aurora Hospital). Blood smears obtained there showed *Plasmodium* parasites that were considered atypical, and the laboratory reported suspicion of a co-infection (*P. falciparum* and *P. malariae*) (Figure). The IV quinine dihydrochloride was replaced with oral quinine hydrochloride, and doxycycline was continued. During treatment, the patient experienced an attack of hypoglycemia (electrocardiogram and blood pressure was normal during this attack), transient mild visual and hearing loss, and transient lymphopenia (a low of 0.46 × 10⁹/L). He received quinine hydrochloride and doxycycline for a total of 10 days.

Because identification of the *Plasmodium* species was difficult, a blood sample was drawn for PCR analysis on day 2 of hospitalization. First, a nested PCR was performed according to a standard protocol with rOval and rFLU2 primers (template DNA purified in Basel from 200 μL of erythrocytes by QIAamp DNA Mini Blood Kit (QIAGEN,

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DOI: 10.3201/eid1409.080170

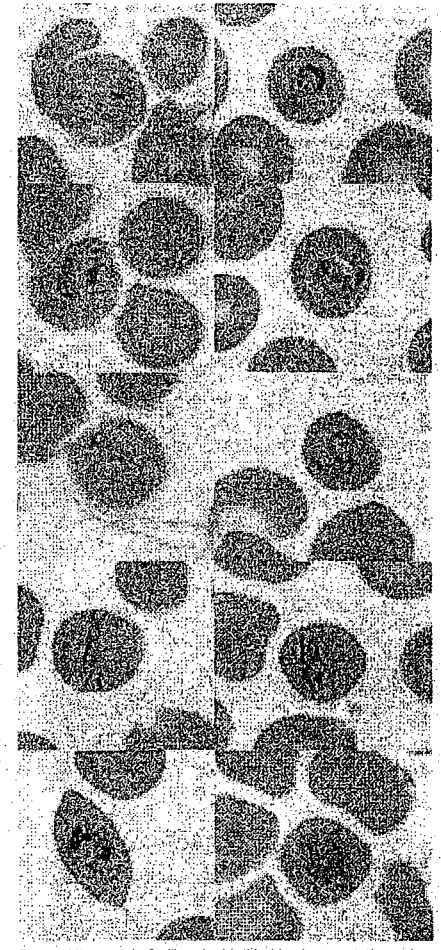

Figure. Microscopic findings in the thin blood smears of a patient with *Plasmodium knowlesi* malaria. Early ring forms are shown in the first row, later trophozoites in the second and third rows, trophozoites resembling band forms in the fourth row, and putative early gametocytes or schizonts in the fifth row. Size of the infected erythrocytes is normal. Antimalarial medications, given 8 hours before the blood shown in the smear was drawn, could have affected morphology. (Original magnification ×1,000.)

Helsinki, Finland) (*13*,*14*), but the reaction did not yield any amplification product. Nested PCR was repeated with an alternative primer pair (rPLU6 and rPLU2) (*14*) derived from a conserved region of the 18S rRNA marker gene, and an amplicon was obtained. Failure of PCR amplification has been reported for some *P. ovale* isolates (*15*); therefore, a *P. ovale* infection was suspected, and the patient was given primaquine phosphate for 14 days as an outpatient to eradicate possible liver hypnozoites. The PCR product was subjected to direct nucleotide sequencing (GenBank accession no. FJ009511) and found to be identical to 2 *P. knowlesi* sequences previously submitted to GenBank, 1 human isolate from Malaysian Borneo (AY327556) and a *Macaca mulatta* isolate from Columbia (U72542). Six other published *P. knowlesi* sequences differ from our sequence only by 1 nucleotide (99% identity). In contrast, a number of differences were seen between our sequence and the *P. ovale* sequences (*15*). The sequence from our case showed only 50% identity to the ovale primer; therefore, we concluded that our patient was infected with *P. knowlesi*. During the 12-month follow-up period, the patient showed no signs of relapse.

Conclusions

We suggest that *P. knowlesi* infection should be considered in malaria patients who have a history of a travel to forested areas in Southeast Asia, especially if *P. malariae* malaria is diagnosed or atypical plasmodia are seen with microscopy. The asexual stages of various species of *P. knowlesi* can easily be misidentified as *P. malariae* in light microscopic examination (Figure) (*3*,*7*,*10*). Because most laboratories diagnose malaria by light microscope examination only, numerous cases of *P. knowlesi* malaria may have been misdiagnosed as ordinary *P. malariae* malaria; monkey malaria may be more widespread among humans than was previously thought. As the disease is potentially dangerous, a proper identification of the malaria species is crucial. If PCR assays for malaria detection are used, PCR primers specific for *P. knowlesi* (*3*) should be included to provide valuable diagnostic information.

P. knowlesi has established itself as the fifth species of *Plasmodium* that causes human malaria (*3*,*7*,*12*). Because the disease is potentially life-threatening in humans, laboratory clinicians and physicians (especially those taking care of travelers) should become more aware of this disease; it is easily misdiagnosed as a less severe form of malaria.

Acknowledgments

We thank the patient for allowing us to publish his case, Heli Siikamäki for helpful discussions, and personnel of the Unit of Parasitology, Helsinki University Central Hospital Laboratory, for recognizing the atypical nature of *Plasmodium* parasites in the patient's thin blood smears.

The research of T.S.J. is financially supported by the Academy of Finland (projects 201506 and 202529), the Helsinki University Central Hospital Funds, and the Sigrid Juselius Foundation; the work of A.K. is supported by the Finnish Medical Foundation and the special Finnish governmental subsidy for health sciences research.

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References

1. Rich SM, Ayala FJ. Progress in malaria research: the case for phylogenetics. Adv Parasitol. 2003;54:255–80. DOI: 10.1016/S0065-308X(03)54005-2
2. Garnham PCC. Malaria parasites and other haemosporidia. Oxford (UK): Blackwell Scientific Publications; 1966.
3. Singh B, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired *Plasmodium knowlesi* infections in human beings. Lancet. 2004;363:1017–24. DOI: 10.1016/S0140-6736(04)15836-4
4. Zhu HM, Li J, Zheng H. Human natural infection of *Plasmodium knowlesi* [in Chinese]. Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi. 2006;24:70–1.
5. Jongwutiwes S, Putaporntip C, Iwasaki T, Sata T, Kanbara H. Naturally acquired *Plasmodium knowlesi* malaria in human, Thailand. Emerg Infect Dis. 2004;10:2211–3.
6. Fong YL, Cadigan FC, Coatney GR. A presumptive case of naturally occurring *Plasmodium knowlesi* malaria in man in Malaysia. Trans R Soc Trop Med Hyg. 1971;65:839–40. DOI: 10.1016/0035-9203(71)90103-9
7. Cox-Singh J, Davis TM, Lee KS, Shamsul SS, Matusop A, Ratnam S, et al. *Plasmodium knowlesi* malaria in humans is widely distributed and potentially life threatening. Clin Infect Dis. 2008;46:165–71. DOI: 10.1086/524888
8. Luchavez J, Espino F, Curameng P, Espina R, Bell D, Chiodini P, et al. Human infections with *Plasmodium knowlesi*, the Philippines. Emerg Infect Dis. 2008;14:811–3.
9. Ng OT, Ooi EE, Lee CC, Lee PJ, Ng LC, Pei SW, et al. Naturally acquired human *Plasmodium knowlesi* infection, Singapore. Emerg Infect Dis. 2008;14:814–6.
10. Knowles R, Das Gupta BM. A study of monkey malaria and its experimental transmission to man (preliminary report). Ind Med Gaz. 1932;67:301–20.
11. Chin W, Contacos PG, Coatney GR, Kimball HR. A naturally acquired quotidian-type malaria in man transferable to monkeys. Science. 1965;149:865. DOI: 10.1126/science.149.3686.865
12. Fleck F. Monkey malaria could represent a new human strain. Bull World Health Organ 2004;82:392–3. DOI: 10.1590/S0042-96862004000500017
13. Snounou G, Singh B. Nested PCR analysis of *Plasmodium* parasites. Methods Mol Med. 2002;72:189–203.
14. Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, et al. High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction. Mol Biochem Parasitol. 1993;61:315–20. DOI: 10.1016/0166-6851(93)90077-B
15. Win TT, Jalloh A, Tantular IS, Tsuboi T, Ferreira MU, Kimura M, et al. Molecular analysis of *Plasmodium ovale* variants. Emerg Infect Dis. 2004;10:1235–40.

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別紙様式第2-1

No. 22

医薬品 研究報告 調査報告書

識別番号・報告回数			報告日	第一報入手日	新医薬品等の区分	総合機構処理欄
				2008. 9. 18	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	野崎一朗, 浜口毅, 篠原もえ子, 中村好一, 北本哲之, 佐藤猛, 水澤英洋, 森若文雄, 志賀裕正, 三條伸夫, 黒岩義之, 西澤正豊, 武田雅俊, 葛原茂樹, 黒田重利, 村井弘之, 村山繁雄, 立石潤, 山田正仁. 2008年プリオン研究会; 2008 Aug 29-30; 新得町.	公表国 日本	
販売名(企業名)	解凍赤血球濃厚液「日赤」(日本赤十字社) 照射解凍赤血球濃厚液「日赤」(日本赤十字社) 解凍赤血球-LR「日赤」(日本赤十字社) 照射解凍赤血球-LR「日赤」(日本赤十字社)					

研究報告の概要

○わが国におけるヒトのプリオン病の発症状況:最近9年間のサーベイランスデータ

【背景・目的】わが国のプリオン病の病型は多彩であり、その発症動向を把握することは重要な課題と考えられる。

【方法】現行のサーベイランスシステムが開始された1999年4月から2008年2月までの9年間に、プリオン病の疑いとして情報収集された1339例を検討した結果、プリオン病と判定された症例について、その内訳、発症状況などを検討した。

【結果】1069例がプリオン病と判定された。プリオン病の発症数は、年間120例前後で推移していた。病型別では孤発性CJDが821例(76.8%)、遺伝性プリオン病が171例(16.0%)、硬膜移植後CJD74例(6.9%)、変異型CJD1例(0.1%)、分類不能2例(0.2%)であった。プリオン病の剖検率については、全体で19.1%と欧米諸国の平均よりも著明に低く、最も多く検索されていた硬膜移植後CJDにおいても37%と低かった。病型が判明している孤発性CJD32例では、MM1が最も多く、次にMM2が皮質型、視床型ほぼ同数で欧米と比較すると多い結果となった。MV1、VV1は1例も確認されなかった。遺伝性プリオン病の変異別頻度はV180I、P102L、E200K、M232R他の順で、欧米諸国のデータとは異なっていた。硬膜移植後CJDの発生は2002年以降減少傾向にあり、現在までに132例が確認された。変異型CJDに関しては、2001年に発症した1例のみであった。

【結論】わが国のプリオン病剖検率は欧米諸国に比較し著明に低率であった。孤発性CJDについては、わが国では欧米に比較してMM2型が多かった。硬膜移植後CJDが多発しているが、2002年以降はその発生は減少傾向であった。遺伝性プリオン病の変異別頻度は欧米諸国の割合と著しく異なっていた。

使用上の注意記載状況・その他参考事項等

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

血液を介するウイルス、細菌、原虫等の感染
vCJD等の伝播のリスク

報告企業の意見

CJDサーベイランス委員会による調査では過去9年間に日本国内で1069例がプリオン病と判定された。また、我が国では剖検率が欧米諸国より著明に低く、病型は欧米諸国と大きく異なっているとの報告である。

今後の対応

日本赤十字社は、vCJDの血液を介する感染防止の目的から、献血時に過去の海外渡航歴(旅行及び居住)を確認し、欧州36ヶ国に一定期間滞在したドナーを無期限に献血延期としている。また、英国滞在歴を有するvCJD患者が国内で発生したことから、平成17年6月1日より1980～96年に1日以上の英国滞在歴のある方からの献血を制限している。加えて、CJDの感染防止の目的から、プリオン病家族歴、硬膜移植歴について問診を行い、該当するドナーを無期限に献血延期としている。今後もCJD等プリオン病に関する新たな知見及び情報の収集に努める。

(13)

JRC2008T-060

Poster-33

わが国におけるヒトのプリオン病の発症状況：最近9年間のサーベイランスデータ

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【背景・目的】わが国では、通常の孤発性 Creutzfeldt-Jakob 病（CJD）、硬膜移植後 CJD に加え、ウシ海綿状脳症からの感染が疑われる変異型 CJD も確認されている。プリオン病の病型は多彩であり、その発症動向を把握することは重要な課題と考えられる。

【方法】「プリオン病及び遅発性ウイルス感染症に関する調査研究班」・CJD サーベイランス委員会による現行のサーベイランスシステムは 1999 年 4 月より開始され、2008 年 2 月までの 9 年間にプリオン病の疑いとして情報収集された 1339 例が検討された。CJD サーベイランス委員会での検討の結果、プリオン病と判定された症例について、その内訳、発症状況などを検討した。

【結果】1069 例がプリオン病と判定された。プリオン病の発症数については、2007 年はまだ情報収集不足で少ないが、それ以外は年間 120 例前後で推移していた。病型別では孤発性 CJD が 821 例（76.8%）、遺伝性プリオン病が 171 例(16.0%)、硬膜移植後 CJD 74 例（6.9%）、変異型 CJD 1 例（0.1%）、分類不能 2 例(0.2%)であった。プリオン病の剖検率については、全体で 19.1%と欧米諸国の平均よりも著明に低かった。分類別では、最も多く検索されていたのは硬膜移植後 CJD であったが、それでも 37%と低い割合にとどまっていた。孤発性 CJD におけるプリオン蛋白遺伝子コドン 129 多型とプロテアーゼ抵抗性プリオン蛋白ウェスタンブロット解析パターンの組み合わせによる病型が判明しているものは 32 例であった。最も多いのは MM1 であったが、次に MM2 が皮質型、視床型ほぼ同数あり、欧米のデータと比較すると多い結果となった。MV1、VV1 は 1 例も確認されなかった。遺伝性プリオン病の変異別頻度は V180I、P102L、E200K、M232R 他の順であった。欧米諸国のデータと比較すると、日本で 4 割を占める V180I は欧米諸国ではまれで、4 番目に多い M232R については欧米では 1 例も認められなかった。一方欧米で 2 番目に多い V210I はわが国では確認されなかった。硬膜移植後 CJD の発生は 2002 年以降減少傾向にあり、現在までに 132 例が確認された。変異型 CJD に関しては、2001 年に発症した 1 例のみであった。

【結論】わが国のプリオン病剖検率は欧米諸国に比較し著明に低率であった。孤発性 CJD については、わが国では欧米に比較して MM2 型が多かったが、剖検率自体が低く非典型例が多く剖検されている可能性が考えられた。硬膜移植後 CJD が多発しているが、2002 年以降はその発生は減少傾向であった。遺伝性プリオン病の変異別頻度は V180I、P102L、E200K、M232R 他の順で、これは欧米諸国の割合と著しく異なっていた。